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(54) Title: PLANT BIOCHEMISTRY-RELATED GENES

(57) Abstract: Recombinant polynucleotides and methods for modifying the phenotype of a plant are provided. In particular, the phenotype that is being modified is a plant's biochemical characteristic.

## PLANT BIOCHEMISTRY-RELATED GENES

### RELATED APPLICATION INFORMATION

The present invention claims the benefit from US Provisional Patent Application Serial  
5 Nos. 60/166,228 filed November 17, 1999 and 60/197,899 filed April 17, 2000 and "Plant Trait  
Modification III" filed August 22, 2000.

### FIELD OF THE INVENTION

This invention relates to the field of plant biology. More particularly, the present  
invention pertains to compositions and methods for phenotypically modifying a plant.

### 10 BACKGROUND OF THE INVENTION

Transcription factors can modulate gene expression, either increasing or decreasing  
(inducing or repressing) the rate of transcription. This modulation results in differential levels of  
gene expression at various developmental stages, in different tissues and cell types, and in  
response to different exogenous (e.g., environmental) and endogenous stimuli throughout the life  
15 cycle of the organism.

Because transcription factors are key controlling elements of biological pathways,  
altering the expression levels of one or more transcription factors can change entire biological  
pathways in an organism. For example, manipulation of the levels of selected transcription  
factors may result in increased expression of economically useful proteins or metabolic chemicals  
20 in plants or to improve other agriculturally relevant characteristics. Conversely, blocked or  
reduced expression of a transcription factor may reduce biosynthesis of unwanted compounds or  
remove an undesirable trait. Therefore, manipulating transcription factor levels in a plant offers  
tremendous potential in agricultural biotechnology for modifying a plant's traits.

The present invention provides novel transcription factors useful for modifying a plant's  
25 phenotype in desirable ways, such as modifying a plant's biochemical traits.

### SUMMARY OF THE INVENTION

In a first aspect, the invention relates to a recombinant polynucleotide comprising a  
nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a  
polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-22, or a  
30 complementary nucleotide sequence thereof; (b) a nucleotide sequence encoding a polypeptide  
comprising a conservatively substituted variant of a polypeptide of (a); (c) a nucleotide sequence  
comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-22, or a  
complementary nucleotide sequence thereof; (d) a nucleotide sequence comprising silent

substitutions in a nucleotide sequence of (c); (e) a nucleotide sequence which hybridizes under stringent conditions over substantially the entire length of a nucleotide sequence of one or more of: (a), (b), (c), or (d); (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e); (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide having a biological activity that modifies a plant's biochemical characteristic; (h) a nucleotide sequence having at least 31% sequence identity to a nucleotide sequence of any of (a)-(g); (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g); (j) a nucleotide sequence which encodes a polypeptide having at least 31% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-22; (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-22; and (l) a nucleotide sequence which encodes a conserved domain of a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-22. The recombinant polynucleotide may further comprise a constitutive, inducible, or tissue-active promoter operably linked to the nucleotide sequence. The invention also relates to compositions comprising at least two of the above described polynucleotides.

In a second aspect, the invention is an isolated or recombinant polypeptide comprising a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotide described above.

In another aspect, the invention is a transgenic plant comprising one or more of the above described recombinant polynucleotides. In yet another aspect, the invention is a plant with altered expression levels of a polynucleotide described above or a plant with altered expression or activity levels of an above described polypeptide. Further, the invention is a plant lacking a nucleotide sequence encoding a polypeptide described above. The plant may be a soybean, wheat, corn, potato, cotton, rice, oilseed rape, sunflower, alfalfa, sugarcane, turf, banana, blackberry, blueberry, strawberry, raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits, or vegetable brassicas plant.

In a further aspect, the invention relates to a cloning or expression vector comprising the isolated or recombinant polynucleotide described above or cells comprising the cloning or expression vector.

In yet a further aspect, the invention relates to a composition produced by incubating a polynucleotide of the invention with a nuclease, a restriction enzyme, a polymerase; a polymerase and a primer; a cloning vector, or with a cell.

Furthermore, the invention relates to a method for producing a plant having a modified  
5 biochemical trait. The method comprises altering the expression of an isolated or recombinant polynucleotide of the invention or altering the expression or activity of a polypeptide of the invention in a plant to produce a modified plant, and selecting the modified plant for a modified biochemical trait.

In another aspect, the invention relates to a method of identifying a factor that is  
10 modulated by or interacts with a polypeptide encoded by a polynucleotide of the invention. The method comprises expressing a polypeptide encoded by the polynucleotide in a plant; and identifying at least one factor that is modulated by or interacts with the polypeptide. In one embodiment the method for identifying modulating or interacting factors is by detecting binding by the polypeptide to a promoter sequence, or by detecting interactions between an additional  
15 protein and the polypeptide in a yeast two hybrid system, or by detecting expression of a factor by hybridization to a microarray, subtractive hybridization or differential display.

In yet another aspect, the invention is a method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest. The method comprises  
20 placing the molecule in contact with a plant comprising the polynucleotide or polypeptide encoded by the polynucleotide of the invention and monitoring one or more of the expression level of the polynucleotide in the plant, the expression level of the polypeptide in the plant, and modulation of an activity of the polypeptide in the plant.

In yet another aspect, the invention relates to an integrated system, computer or computer readable medium comprising one or more character strings corresponding to a polynucleotide of  
25 the invention, or to a polypeptide encoded by the polynucleotide. The integrated system, computer or computer readable medium may comprise a link between one or more sequence strings to a modified plant biochemical trait.

In yet another aspect, the invention is a method for identifying a sequence similar or homologous to one or more polynucleotides of the invention, or one or more polypeptides  
30 encoded by the polynucleotides. The method comprises providing a sequence database; and, querying the sequence database with one or more target sequences corresponding to the one or more polynucleotides or to the one or more polypeptides to identify one or more sequence members of the database that display sequence similarity or homology to one or more of the one or more target sequences.



The method may further comprise of linking the one or more of the polynucleotides of the invention, or encoded polypeptides, to a modified plant biochemical phenotype.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides a table of exemplary polynucleotide and polypeptide sequences of the invention. The table includes from left to right for each sequence: the SEQ ID No., the internal code reference number (GID), whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences.

Figure 2 provides a table of exemplary sequences that are homologous to other sequences provided in the Sequence Listing and that are derived from *Arabidopsis thaliana*. The table includes from left to right: the SEQ ID No., the internal code reference number (GID), identification of the homologous sequence, whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences.

Figure 3 provides a table of exemplary sequences that are homologous to the sequences provided in Figures 1 and 2 and that are derived from plants other than *Arabidopsis thaliana*. The table includes from left to right: the SEQ ID No., the internal code reference number (GID), the unique GenBank sequence ID No. (NID), the probability that the comparison was generated by chance (P-value), and the species from which the homologous gene was identified.

#### DETAILED DESCRIPTION

The present invention relates to polynucleotides and polypeptides, e.g. for modifying phenotypes of plants.

In particular, the polynucleotides or polypeptides are useful for modifying traits associated with a plant's biochemical characteristic when the expression levels of the polynucleotides or expression levels or activity levels of the polypeptides are altered.

The polynucleotides of the invention encode plant transcription factors. The plant transcription factors are derived, e.g., from *Arabidopsis thaliana* and can belong, e.g., to one or more of the following transcription factor families: the AP2 (APETALA2) domain transcription factor family (Riechmann and Meyerowitz (1998) *J. Biol. Chem.* 379:633-646); the MYB transcription factor family (Martin and Paz-Ares (1997) *Trends Genet.* 13:67-73); the MADS domain transcription factor family (Riechmann and Meyerowitz (1997) *J. Biol. Chem.* 378:1079-1101); the WRKY protein family (Ishiguro and Nakamura (1994) *Mol. Gen. Genet.* 244:563-571); the ankyrin-repeat protein family (Zhang et al. (1992) *Plant Cell* 4:1575-1588); the

miscellaneous protein (MISC) family (Kim et al. (1997) Plant J. 11:1237-1251); the zinc finger protein (Z) family (Klug and Schwabe (1995) FASEB J. 9: 597-604); the homeobox (HB) protein family (Duboule (1994) Guidebook to the Homeobox Genes, Oxford University Press); the CAAT-element binding proteins (Forsburg and Guarente (1989) Genes Dev. 3:1166-1178); the squamosa promoter binding proteins (SPB) (Klein et al. (1996) Mol. Gen. Genet. 1996 250:7-16); the NAM protein family; the IAA/AUX proteins (Rouse et al. (1998) Science 279:1371-1373); the HLH/MYC protein family (Littlewood et al. (1994) Prot. Profile 1:639-709); the DNA-binding protein (DBP) family (Tucker et al. (1994) EMBO J. 13:2994-3002); the bZIP family of transcription factors (Foster et al. (1994) FASEB J. 8:192-200); the BPF-1 protein (Box P-binding factor) family (da Costa e Silva et al. (1993) Plant J. 4:125-135); and the golden protein (GLD) family (Hall et al. (1998) Plant Cell 10:925-936).

In addition to methods for modifying a plant phenotype by employing one or more polynucleotides and polypeptides of the invention described herein, the polynucleotides and polypeptides of the invention have a variety of additional uses. These uses include their use in the recombinant production (i.e., expression) of proteins; as regulators of plant gene expression, as diagnostic probes for the presence of complementary or partially complementary nucleic acids (including for detection of natural coding nucleic acids); as substrates for further reactions, e.g., mutation reactions, PCR reactions, or the like, or as substrates for cloning e.g., including digestion or ligation reactions, and for identifying exogenous or endogenous modulators of the transcription factors.

### DEFINITIONS

A "polynucleotide" is a nucleic acid sequence comprising a plurality of polymerized nucleotide residues, e.g., at least about 15 consecutive polymerized nucleotide residues, optionally at least about 30 consecutive nucleotides, at least about 50 consecutive nucleotides. In many instances, a polynucleotide comprises a nucleotide sequence encoding a polypeptide (or protein) or a domain or fragment thereof. Additionally, the polynucleotide may comprise a promoter, an intron, an enhancer region, a polyadenylation site, a translation initiation site, 5' or 3' untranslated regions, a reporter gene, a selectable marker, or the like. The polynucleotide can be single stranded or double stranded DNA or RNA. The polynucleotide optionally comprises modified bases or a modified backbone. The polynucleotide can be, e.g., genomic DNA or RNA, a transcript (such as an mRNA), a cDNA, a PCR product, a cloned DNA, a synthetic DNA or RNA, or the like. The polynucleotide can comprise a sequence in either sense or antisense orientations.

A "recombinant polynucleotide" is a polynucleotide that is not in its native state, e.g., the polynucleotide comprises a nucleotide sequence not found in nature, or the polynucleotide is in a context other than that in which it is naturally found, e.g., separated from nucleotide sequences with which it typically is in proximity in nature, or adjacent (or contiguous with) nucleotide sequences with which it typically is not in proximity. For example, the sequence at issue can be cloned into a vector, or otherwise recombined with one or more additional nucleic acid.

An "isolated polynucleotide" is a polynucleotide whether naturally occurring or recombinant, that is present outside the cell in which it is typically found in nature, whether purified or not. Optionally, an isolated polynucleotide is subject to one or more enrichment or purification procedures, e.g., cell lysis, extraction, centrifugation, precipitation, or the like.

A "recombinant polypeptide" is a polypeptide produced by translation of a recombinant polynucleotide. An "isolated polypeptide," whether a naturally occurring or a recombinant polypeptide, is more enriched in (or out of) a cell than the polypeptide in its natural state in a wild type cell, e.g., more than about 5% enriched, more than about 10% enriched, or more than about 20%, or more than about 50%, or more, enriched, i.e., alternatively denoted: 105%, 110%, 120%, 150% or more, enriched relative to wild type standardized at 100%. Such an enrichment is not the result of a natural response of a wild type plant. Alternatively, or additionally, the isolated polypeptide is separated from other cellular components with which it is typically associated, e.g., by any of the various protein purification methods herein.

The term "transgenic plant" refers to a plant that contains genetic material, not found in a wild type plant of the same species, variety or cultivar. The genetic material may include a transgene, an insertional mutagenesis event (such as by transposon or T-DNA insertional mutagenesis), an activation tagging sequence, a mutated sequence, a homologous recombination event or a sequence modified by chimeraplasty. Typically, the foreign genetic material has been introduced into the plant by human manipulation.

A transgenic plant may contain an expression vector or cassette. The expression cassette typically comprises a polypeptide-encoding sequence operably linked (i.e., under regulatory control of) to appropriate inducible or constitutive regulatory sequences that allow for the expression of polypeptide. The expression cassette can be introduced into a plant by transformation or by breeding after transformation of a parent plant. A plant refers to a whole plant as well as to a plant part, such as seed, fruit, leaf, or root, plant tissue, plant cells or any other plant material, e.g., a plant explant, as well as to progeny thereof, and to *in vitro* systems that mimic biochemical or cellular components or processes in a cell.

The phrase "ectopically expression or altered expression" in reference to a polynucleotide indicates that the pattern of expression in, e.g., a transgenic plant or plant tissue, is different from the expression pattern in a wild type plant or a reference plant of the same species. For example, the polynucleotide or polypeptide is expressed in a cell or tissue type other than a cell or tissue  
5 type in which the sequence is expressed in the wild type plant, or by expression at a time other than at the time the sequence is expressed in the wild type plant, or by a response to different inducible agents, such as hormones or environmental signals, or at different expression levels (either higher or lower) compared with those found in a wild type plant. The term also refers to altered expression patterns that are produced by lowering the levels of expression to below the  
10 detection level or completely abolishing expression. The resulting expression pattern can be transient or stable, constitutive or inducible. In reference to a polypeptide, the term "ectopic expression or altered expression" further may relate to altered activity levels resulting from the interactions of the polypeptides with exogenous or endogenous modulators or from interactions with factors or as a result of the chemical modification of the polypeptides.

15 The term "fragment" or "domain," with respect to a polypeptide, refers to a subsequence of the polypeptide. In some cases, the fragment or domain, is a subsequence of the polypeptide which performs at least one biological function of the intact polypeptide in substantially the same manner, or to a similar extent, as does the intact polypeptide. For example, a polypeptide fragment can comprise a recognizable structural motif or functional domain such as a DNA  
20 binding domain that binds to a DNA promoter region, an activation domain or a domain for protein-protein interactions. Fragments can vary in size from as few as 6 amino acids to the full length of the intact polypeptide, but are preferably at least about 30 amino acids in length and more preferably at least about 60 amino acids in length. In reference to a nucleotide sequence, "a fragment" refers to any subsequence of a polynucleotide, typically, of at least consecutive about  
25 15 nucleotides, preferably at least about 30 nucleotides, more preferably at least about 50, of any of the sequences provided herein.

The term "trait" refers to a physiological, morphological, biochemical or physical characteristic of a plant or particular plant material or cell. In some instances, this characteristic is visible to the human eye, such as seed or plant size, or can be measured by available  
30 biochemical techniques, such as the protein, starch or oil content of seed or leaves or by the observation of the expression level of genes, e.g., by employing Northern analysis, RT-PCR, microarray gene expression assays or reporter gene expression systems, or by agricultural observations such as stress tolerance, yield or pathogen tolerance.

“Trait modification” refers to a detectable difference in a characteristic in a plant ectopically expressing a polynucleotide or polypeptide of the present invention relative to a plant not doing so, such as a wild type plant. In some cases, the trait modification can be evaluated quantitatively. For example, the trait modification can entail at least about a 2% increase or  
5 decrease in an observed trait (difference), at least a 5% difference, at least about a 10% difference, at least about a 20% difference, at least about a 30%, at least about a 50%, at least about a 70%, or at least about a 100%, or an even greater difference. It is known that there can be a natural variation in the modified trait. Therefore, the trait modification observed entails a change of the normal distribution of the trait in the plants compared with the distribution  
10 observed in wild type plant.

Trait modifications of particular interest include those to seed (such as embryo or endosperm), fruit, root, flower, leaf, stem, shoot, seedling or the like, including: enhanced tolerance to environmental conditions including freezing, chilling, heat, drought, water saturation, radiation and ozone; improved tolerance to microbial, fungal or viral diseases; improved  
15 tolerance to pest infestations, including nematodes, mollicutes, parasitic higher plants or the like; decreased herbicide sensitivity; improved tolerance of heavy metals or enhanced ability to take up heavy metals; improved growth under poor photoconditions (e.g., low light and/or short day length), or changes in expression levels of genes of interest. Other phenotype that can be modified relate to the production of plant metabolites, such as variations in the production of  
20 taxol, tocopherol, tocotrienol, sterols, phytosterols, vitamins, wax monomers, anti-oxidants, amino acids, lignins, cellulose, tannins, prenolipids (such as chlorophylls and carotenoids), glucosinolates, and terpenoids, enhanced or compositionally altered protein or oil production (especially in seeds), or modified sugar (insoluble or soluble) and/or starch composition.

Physical plant characteristics that can be modified include cell development (such as the number  
25 of trichomes), fruit and seed size and number, yields of plant parts such as stems, leaves and roots, the stability of the seeds during storage, characteristics of the seed pod (e.g., susceptibility to shattering), root hair length and quantity, internode distances, or the quality of seed coat. Plant growth characteristics that can be modified include growth rate, germination rate of seeds, vigor of plants and seedlings, leaf and flower senescence, male sterility, apomixis, flowering time,  
30 flower abscission, rate of nitrogen uptake, biomass or transpiration characteristics, as well as plant architecture characteristics such as apical dominance, branching patterns, number of organs, organ identity, organ shape or size.

### POLYPEPTIDES AND POLYNUCLEOTIDES OF THE INVENTION

The present invention provides, among other things, transcription factors (TFs), and transcription factor homologue polypeptides, and isolated or recombinant polynucleotides encoding the polypeptides. These polypeptides and polynucleotides may be employed to modify  
5 a plant's biochemical characteristic.

Exemplary polynucleotides encoding the polypeptides of the invention were identified in the *Arabidopsis thaliana* GenBank database using publicly available sequence analysis programs and parameters. Sequences initially identified were then further characterized to identify sequences comprising specified sequence strings corresponding to sequence motifs present in  
10 families of known transcription factors. Polynucleotide sequences meeting such criteria were confirmed as transcription factors.

Additional polynucleotides of the invention were identified by screening *Arabidopsis thaliana* and/or other plant cDNA libraries with probes corresponding to known transcription factors under low stringency hybridization conditions. Additional sequences, including full  
15 length coding sequences were subsequently recovered by the rapid amplification of cDNA ends (RACE) procedure, using a commercially available kit according to the manufacturer's instructions. Where necessary, multiple rounds of RACE are performed to isolate 5' and 3' ends. The full length cDNA was then recovered by a routine end-to-end polymerase chain reaction (PCR) using primers specific to the isolated 5' and 3' ends. Exemplary sequences are provided in  
20 the Sequence Listing.

The polynucleotides of the invention were ectopically expressed in overexpressor or knockout plants and changes in the biochemical characteristics of the plants were observed. Therefore, the polynucleotides and polypeptides can be employed to improve the biochemical characteristics of plants:

#### Making polynucleotides

The polynucleotides of the invention include sequences that encode transcription factors and transcription factor homologue polypeptides and sequences complementary thereto, as well as unique fragments of coding sequence, or sequence complementary thereto. Such  
25 polynucleotides can be, e.g., DNA or RNA, e.g., mRNA, cRNA, synthetic RNA, genomic DNA, cDNA synthetic DNA, oligonucleotides, etc. The polynucleotides are either double-stranded or  
30 single-stranded, and include either, or both sense (i.e., coding) sequences and antisense (i.e., non-coding, complementary) sequences. The polynucleotides include the coding sequence of a transcription factor, or transcription factor homologue polypeptide, in isolation, in combination with additional coding sequences (e.g., a purification tag, a localization signal, as a fusion-

protein, as a pre-protein, or the like), in combination with non-coding sequences (e.g., introns or inteins, regulatory elements such as promoters, enhancers, terminators, and the like), and/or in a vector or host environment in which the polynucleotide encoding a transcription factor or transcription factor homologue polypeptide is an endogenous or exogenous gene.

- 5           A variety of methods exist for producing the polynucleotides of the invention. Procedures for identifying and isolating DNA clones are well known to those of skill in the art, and are described in, e.g., Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology volume 152 Academic Press, Inc., San Diego, CA ("Berger"); Sambrook et al., Molecular Cloning - A Laboratory Manual (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989 ("Sambrook") and Current Protocols in Molecular Biology, F.M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (supplemented through 2000) ("Ausubel").

- 15           Alternatively, polynucleotides of the invention, can be produced by a variety of in vitro amplification methods adapted to the present invention by appropriate selection of specific or degenerate primers. Examples of protocols sufficient to direct persons of skill through in vitro amplification methods, including the polymerase chain reaction (PCR) the ligase chain reaction (LCR), Qbeta-replicase amplification and other RNA polymerase mediated techniques (e.g., NASBA), e.g., for the production of the homologous nucleic acids of the invention are found in Berger, Sambrook, and Ausubel, as well as Mullis et al., (1987) PCR Protocols A Guide to Methods and Applications (Innis et al. eds) Academic Press Inc. San Diego, CA (1990) (Innis). Improved methods for cloning in vitro amplified nucleic acids are described in Wallace et al., U.S. Pat. No. 5,426,039. Improved methods for amplifying large nucleic acids by PCR are summarized in Cheng et al. (1994) Nature 369: 684-685 and the references cited therein, in which PCR amplicons of up to 40kb are generated. One of skill will appreciate that essentially any RNA can be converted into a double stranded DNA suitable for restriction digestion, PCR expansion and sequencing using reverse transcriptase and a polymerase. See, e.g., Ausubel, Sambrook and Berger, *all supra*.

- 25           Alternatively, polynucleotides and oligonucleotides of the invention can be assembled from fragments produced by solid-phase synthesis methods. Typically, fragments of up to approximately 100 bases are individually synthesized and then enzymatically or chemically ligated to produce a desired sequence, e.g., a polynucleotide encoding all or part of a transcription factor. For example, chemical synthesis using the phosphoramidite method is described, e.g., by Beaucage et al. (1981) Tetrahedron Letters 22:1859-69; and Matthes et al. (1984) EMBO J. 3:801-5. According to such methods, oligonucleotides are synthesized, purified,

annealed to their complementary strand, ligated and then optionally cloned into suitable vectors. And if so desired, the polynucleotides and polypeptides of the invention can be custom ordered from any of a number of commercial suppliers.

#### HOMOLOGOUS SEQUENCES

5           Sequences homologous, i.e., that share significant sequence identity or similarity, to those provided in the Sequence Listing, derived from *Arabidopsis thaliana* or from other plants of choice are also an aspect of the invention. Homologous sequences can be derived from any plant including monocots and dicots and in particular agriculturally important plant species, including but not limited to, crops such as soybean, wheat, corn, potato, cotton, rice, oilseed rape (including  
10    canola), sunflower, alfalfa, sugarcane and turf; or fruits and vegetables, such as banana, blackberry, blueberry, strawberry, and raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits (such as apple, peach, pear, cherry and plum) and vegetable brassicas (such as broccoli, cabbage,  
15    cauliflower, brussel sprouts and kohlrabi). Other crops, fruits and vegetables whose phenotype can be changed include barley, rye, millet, sorghum, currant, avocado, citrus fruits such as oranges, lemons, grapefruit and tangerines, artichoke, cherries, nuts such as the walnut and peanut, endive, leek, roots, such as arrowroot, beet, cassava, turnip, radish, yam, and sweet potato, and beans. The homologous sequences may also be derived from woody species, such  
20    pine, poplar and eucalyptus.

          Transcription factors that are homologous to the listed sequences will typically share at least about 30% amino acid sequence identity. More closely related transcription factors can share at least about 50%, about 60%, about 65%, about 70%, about 75% or about 80% or about 90% or about 95% or about 98% or more sequence identity with the listed sequences. Factors  
25    that are most closely related to the listed sequences share, e.g., at least about 85%, about 90% or about 95% or more % sequence identity to the listed sequences. At the nucleotide level, the sequences will typically share at least about 40% nucleotide sequence identity, preferably at least about 50%, about 60%, about 70% or about 80% sequence identity, and more preferably about 85%, about 90%, about 95% or about 97% or more sequence identity to one or more of the listed  
30    sequences. The degeneracy of the genetic code enables major variations in the nucleotide sequence of a polynucleotide while maintaining the amino acid sequence of the encoded protein. Conserved domains within a transcription factor family may exhibit a higher degree of sequence



homology, such as at least 65% sequence identity including conservative substitutions, and preferably at least 80% sequence identity.

#### Identifying Nucleic Acids by Hybridization

Polynucleotides homologous to the sequences illustrated in the Sequence Listing can be identified, e.g., by hybridization to each other under stringent or under highly stringent conditions. Single stranded polynucleotides hybridize when they associate based on a variety of well characterized physico-chemical forces, such as hydrogen bonding, solvent exclusion, base stacking and the like. The stringency of a hybridization reflects the degree of sequence identity of the nucleic acids involved, such that the higher the stringency, the more similar are the two polynucleotide strands. Stringency is influenced by a variety of factors, including temperature, salt concentration and composition, organic and non-organic additives, solvents, etc. present in both the hybridization and wash solutions and incubations (and number), as described in more detail in the references cited above.

An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is about 5°C to 20°C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Nucleic acid molecules that hybridize under stringent conditions will typically hybridize to a probe based on either the entire cDNA or selected portions, e.g., to a unique subsequence, of the cDNA under wash conditions of 0.2x SSC to 2.0 x SSC, 0.1% SDS at 50-65° C, for example 0.2 x SSC, 0.1% SDS at 65° C. For identification of less closely related homologues washes can be performed at a lower temperature, e.g., 50° C. In general, stringency is increased by raising the wash temperature and/or decreasing the concentration of SSC.

As another example, stringent conditions can be selected such that an oligonucleotide that is perfectly complementary to the coding oligonucleotide hybridizes to the coding oligonucleotide with at least about a 5-10x higher signal to noise ratio than the ratio for hybridization of the perfectly complementary oligonucleotide to a nucleic acid encoding a transcription factor known as of the filing date of the application. Conditions can be selected such that a higher signal to noise ratio is observed in the particular assay which is used, e.g., about 15x, 25x, 35x, 50x or more. Accordingly, the subject nucleic acid hybridizes to the unique coding oligonucleotide with at least a 2x higher signal to noise ratio as compared to hybridization of the coding oligonucleotide to a nucleic acid encoding known polypeptide. Again, higher signal to noise ratios can be selected, e.g., about 5x, 10x, 25x, 35x, 50x or more. The particular signal will

depend on the label used in the relevant assay, e.g., a fluorescent label, a colorimetric label, a radioactive label, or the like.

Alternatively, transcription factor homologue polypeptides can be obtained by screening an expression library using antibodies specific for one or more transcription factors. With the provision herein of the disclosed transcription factor, and transcription factor homologue nucleic acid sequences, the encoded polypeptide(s) can be expressed and purified in a heterologous expression system (e.g., *E. coli*) and used to raise antibodies (monoclonal or polyclonal) specific for the polypeptide(s) in question. Antibodies can also be raised against synthetic peptides derived from transcription factor, or transcription factor homologue, amino acid sequences.

Methods of raising antibodies are well known in the art and are described in Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York. Such antibodies can then be used to screen an expression library produced from the plant from which it is desired to clone additional transcription factor homologues, using the methods described above. The selected cDNAs can be confirmed by sequencing and enzymatic activity.

#### 15        SEQUENCE VARIATIONS

It will readily be appreciated by those of skill in the art, that any of a variety of polynucleotide sequences are capable of encoding the transcription factors and transcription factor homologue polypeptides of the invention. Due to the degeneracy of the genetic code, many different polynucleotides can encode identical and/or substantially similar polypeptides in addition to those sequences illustrated in the Sequence Listing.

For example, Table 1 illustrates, e.g., that the codons AGC, AGT, TCA, TCC, TCG, and TCT all encode the same amino acid: serine. Accordingly, at each position in the sequence where there is a codon encoding serine, any of the above trinucleotide sequences can be used without altering the encoded polypeptide.

**Table 1**

Amino acids			Codon							
Alanine	Ala	A	GCA	GCC	GCG	GCU				
Cysteine	Cys	C	TGC	TGT						
Aspartic acid	Asp	D	GAC	GAT						
Glutamic acid	Glu	E	GAA	GAG						
Phenylalanine	Phe	F	TTC	TTT						
Glycine	Gly	G	GGA	GGC	GGG	GGT				
Histidine	His	H	CAC	CAT						
Isoleucine	Ile	I	ATA	ATC	ATT					
Lysine	Lys	K	AAA	AAG						
Leucine	Leu	L	TTA	TTG	CTA	CTC	CTG	CTT		
Methionine	Met	M	ATG							
Asparagine	Asn	N	AAC	AAT						
Proline	Pro	P	CCA	CCC	CCG	CCT				
Glutamine	Gln	Q	CAA	CAG						
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGT		
Serine	Ser	S	AGC	AGT	TCA	TCC	TCG	TCT		
Threonine	Thr	T	ACA	ACC	ACG	ACT				
Valine	Val	V	GTA	GTC	GTG	GTT				
Tryptophan	Trp	W	TGG							
Tyrosine	Tyr	Y	TAC	TAT						

Sequence alterations that do not change the amino acid sequence encoded by the polynucleotide are termed "silent" variations. With the exception of the codons ATG and TGG, encoding methionine and tryptophan, respectively, any of the possible codons for the same amino acid can be substituted by a variety of techniques, e.g., site-directed mutagenesis, available in the art. Accordingly, any and all such variations of a sequence selected from the above table are a feature of the invention.

In addition to silent variations, other conservative variations that alter one, or a few amino acids in the encoded polypeptide, can be made without altering the function of the polypeptide, these conservative variants are, likewise, a feature of the invention.

For example, substitutions, deletions and insertions introduced into the sequences provided in the Sequence Listing are also envisioned by the invention. Such sequence modifications can be engineered into a sequence by site-directed mutagenesis (Wu (ed.) Meth. Enzymol. (1993) vol. 217, Academic Press) or the other methods noted below. Amino acid substitutions are typically of single residues; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. In preferred embodiments, deletions or insertions are made in adjacent pairs, e.g., a deletion of two residues or insertion of two residues. Substitutions, deletions, insertions or any combination thereof can be

combined to arrive at a sequence. The mutations that are made in the polynucleotide encoding the transcription factor should not place the sequence out of reading frame and should not create complementary regions that could produce secondary mRNA structure. Preferably, the polypeptide encoded by the DNA performs the desired function.

- 5       Conservative substitutions are those in which at least one residue in the amino acid sequence has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the Table 2 when it is desired to maintain the activity of the protein. Table 2 shows amino acids which can be substituted for an amino acid in a protein and which are typically regarded as conservative substitutions.

10

**Table 2**

Residue	Conservative Substitutions
Ala	Ser
Arg	Lys
Asn	Gln; His
Asp	Glu
Gln	Asn
Cys	Ser
Glu	Asp
Gly	Pro
His	Asn; Gln
Ile	Leu, Val
Leu	Ile; Val
Lys	Arg; Gln
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr; Gly
Thr	Ser; Val
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

Substitutions that are less conservative than those in Table 2 can be selected by picking residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in protein properties will be those in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

#### FURTHER MODIFYING SEQUENCES OF THE INVENTION—MUTATION/ FORCED EVOLUTION

In addition to generating silent or conservative substitutions as noted, above, the present invention optionally includes methods of modifying the sequences of the Sequence Listing. In the methods, nucleic acid or protein modification methods are used to alter the given sequences to produce new sequences and/or to chemically or enzymatically modify given sequences to change the properties of the nucleic acids or proteins.

Thus, in one embodiment, given nucleic acid sequences are modified, e.g., according to standard mutagenesis or artificial evolution methods to produce modified sequences. For example, Ausubel, *supra*, provides additional details on mutagenesis methods. Artificial forced evolution methods are described, e.g., by Stemmer (1994) *Nature* 370:389-391, and Stemmer (1994) *Proc. Natl. Acad. Sci. USA* 91:10747-10751. Many other mutation and evolution methods are also available and expected to be within the skill of the practitioner.

Similarly, chemical or enzymatic alteration of expressed nucleic acids and polypeptides can be performed by standard methods. For example, sequence can be modified by addition of lipids, sugars, peptides, organic or inorganic compounds, by the inclusion of modified nucleotides or amino acids, or the like. For example, protein modification techniques are illustrated in Ausubel, *supra*. Further details on chemical and enzymatic modifications can be found herein. These modification methods can be used to modify any given sequence, or to modify any sequence produced by the various mutation and artificial evolution modification methods noted herein.

Accordingly, the invention provides for modification of any given nucleic acid by mutation, evolution, chemical or enzymatic modification, or other available methods, as well as

for the products produced by practicing such methods, e.g., using the sequences herein as a starting substrate for the various modification approaches.

For example, optimized coding sequence containing codons preferred by a particular prokaryotic or eukaryotic host can be used e.g., to increase the rate of translation or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, as compared with transcripts produced using a non-optimized sequence. Translation stop codons can also be modified to reflect host preference. For example, preferred stop codons for *S. cerevisiae* and mammals are TAA and TGA, respectively. The preferred stop codon for monocotyledonous plants is TGA, whereas insects and *E. coli* prefer to use TAA as the stop codon.

The polynucleotide sequences of the present invention can also be engineered in order to alter a coding sequence for a variety of reasons, including but not limited to, alterations which modify the sequence to facilitate cloning, processing and/or expression of the gene product. For example, alterations are optionally introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, to change codon preference, to introduce splice sites, etc.

Furthermore, a fragment or domain derived from any of the polypeptides of the invention can be combined with domains derived from other transcription factors or synthetic domains to modify the biological activity of a transcription factor. For instance, a DNA binding domain derived from a transcription factor of the invention can be combined with the activation domain of another transcription factor or with a synthetic activation domain. A transcription activation domain assists in initiating transcription from a DNA binding site. Examples include the transcription activation region of VP16 or GAL4 (Moore et al. (1998) Proc. Natl. Acad. Sci. USA 95: 376-381; and Aoyama et al. (1995) Plant Cell 7:1773-1785), peptides derived from bacterial sequences (Ma and Ptashne (1987) Cell 51: 113-119) and synthetic peptides (Giniger and Ptashne, (1987) Nature 330:670-672).

#### EXPRESSION AND MODIFICATION OF POLYPEPTIDES

Typically, polynucleotide sequences of the invention are incorporated into recombinant DNA (or RNA) molecules that direct expression of polypeptides of the invention in appropriate host cells, transgenic plants, in vitro translation systems, or the like. Due to the inherent degeneracy of the genetic code, nucleic acid sequences which encode substantially the same or a functionally equivalent amino acid sequence can be substituted for any listed sequence to provide for cloning and expressing the relevant homologue.

### Vectors, Promoters and Expression Systems

The present invention includes recombinant constructs comprising one or more of the nucleic acid sequences herein. The constructs typically comprise a vector, such as a plasmid, a cosmid, a phage, a virus (e.g., a plant virus), a bacterial artificial chromosome (BAC), a yeast  
 5 artificial chromosome (YAC), or the like, into which a nucleic acid sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available.

10 General texts which describe molecular biological techniques useful herein, including the use and production of vectors, promoters and many other relevant topics, include Berger, Sambrook and Ausubel, *supra*. Any of the identified sequences can be incorporated into a cassette or vector, e.g., for expression in plants. A number of expression vectors suitable for stable transformation of plant cells or for the establishment of transgenic plants have been described  
 15 including those described in Weissbach and Weissbach, (1989) Methods for Plant Molecular Biology, Academic Press, and Gelvin et al., (1990) Plant Molecular Biology Manual, Kluwer Academic Publishers. Specific examples include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, as well as those disclosed by Herrera-Estrella et al. (1983) Nature 303: 209, Bevan (1984) Nucl Acid Res. 12: 8711-8721, Klee (1985) Bio/Technology 3: 637-642,  
 20 for dicotyledonous plants.

Alternatively, non-Ti vectors can be used to transfer the DNA into monocotyledonous plants and cells by using free DNA delivery techniques. Such methods can involve, for example, the use of liposomes, electroporation, microprojectile bombardment, silicon carbide whiskers, and viruses. By using these methods transgenic plants such as wheat, rice (Christou (1991)  
 25 Bio/Technology 9: 957-962) and corn (Gordon-Kamm (1990) Plant Cell 2: 603-618) can be produced. An immature embryo can also be a good target tissue for monocots for direct DNA delivery techniques by using the particle gun (Weeks et al. (1993) Plant Physiol 102: 1077-1084; Vasil (1993) Bio/Technology 10: 667-674; Wan and Lemeaux (1994) Plant Physiol 104: 37-48, and for *Agrobacterium*-mediated DNA transfer (Ishida et al. (1996) Nature Biotech 14: 745-750).

30 Typically, plant transformation vectors include one or more cloned plant coding sequence (genomic or cDNA) under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant transformation vectors typically also contain a promoter (e.g., a regulatory region controlling inducible or constitutive, environmentally-or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start

site, an RNA processing signal (such as intron splice sites), a transcription termination site, and/or a polyadenylation signal.

Examples of constitutive plant promoters which can be useful for expressing the TF sequence include: the cauliflower mosaic virus (CaMV) 35S promoter, which confers  
 5 constitutive, high-level expression in most plant tissues (*see, e.g.,* Odel et al. (1985) Nature 313:810); the nopaline synthase promoter (An et al. (1988) Plant Physiol 88:547); and the octopine synthase promoter (Fromm et al. (1989) Plant Cell 1: 977).

A variety of plant gene promoters that regulate gene expression in response to environmental, hormonal, chemical, developmental signals, and in a tissue-active manner can be  
 10 used for expression of a TF sequence in plants. Choice of a promoter is based largely on the phenotype of interest and is determined by such factors as tissue (e.g., seed, fruit, root, pollen, vascular tissue, flower, carpel, etc.), inducibility (e.g., in response to wounding, heat, cold, drought, light, pathogens, etc.), timing, developmental stage, and the like. Numerous known promoters have been characterized and can favorably be employed to promote expression of a  
 15 polynucleotide of the invention in a transgenic plant or cell of interest. For example, tissue specific promoters include: seed-specific promoters (such as the napin, phaseolin or DC3 promoter described in US Pat. No. 5,773,697), fruit-specific promoters that are active during fruit ripening (such as the dru 1 promoter (US Pat. No. 5,783,393), or the 2A11 promoter (US Pat. No. 4,943,674) and the tomato polygalacturonase promoter (Bird et al. (1988) Plant Mol Biol 11:651),  
 20 root-specific promoters, such as those disclosed in US Patent Nos. 5,618,988, 5,837,848 and 5,905,186, pollen-active promoters such as PTA29, PTA26 and PTA13 (US Pat. No. 5,792,929), promoters active in vascular tissue (Ringli and Keller (1998) Plant Mol Biol 37:977-988), flower-specific (Kaiser et al. (1995) Plant Mol Biol 28:231-243), pollen (Baerson et al. (1994) Plant Mol Biol 26:1947-1959), carpels (Ohl et al. (1990) Plant Cell 2:837-848), pollen and ovules (Baerson et al. (1993) Plant Mol Biol 22:255-267), auxin-inducible promoters (such as that described in  
 25 van der Kop et al. (1999) Plant Mol Biol 39:979-990 or Baumann et al. (1999) Plant Cell 11:323-334), cytokinin-inducible promoter (Guevara-Garcia (1998) Plant Mol Biol 38:743-753), promoters responsive to gibberellin (Shi et al. (1998) Plant Mol Biol 38:1053-1060, Willmott et al. (1998) 38:817-825) and the like. Additional promoters are those that elicit expression in  
 30 response to heat (Ainley et al. (1993) Plant Mol Biol 22: 13-23), light (e.g., the pea rbcS-3A promoter, Kuhlemeier et al. (1989) Plant Cell 1:471, and the maize rbcS promoter, Schaffner and Sheen (1991) Plant Cell 3: 997); wounding (e.g., *wun1*, Siebertz et al. (1989) Plant Cell 1: 961); pathogens (such as the PR-1 promoter described in Buchel et al. (1999) Plant Mol. Biol. 40:387-396, and the PDF1.2 promoter described in Manners et al. (1998) Plant Mol. Biol. 38:1071-80),



and chemicals such as methyl jasmonate or salicylic acid (Gatz et al. (1997) Plant Mol Biol 48: 89-108). In addition, the timing of the expression can be controlled by using promoters such as those acting at senescence (An and Amazon (1995) Science 270: 1986-1988); or late seed development (Odell et al. (1994) Plant Physiol 106:447-458).

5 Plant expression vectors can also include RNA processing signals that can be positioned within, upstream or downstream of the coding sequence. In addition, the expression vectors can include additional regulatory sequences from the 3'-untranslated region of plant genes, e.g., a 3' terminator region to increase mRNA stability of the mRNA, such as the PI-II terminator region of potato or the octopine or nopaline synthase 3' terminator regions.

#### 10 Additional Expression Elements

Specific initiation signals can aid in efficient translation of coding sequences. These signals can include, e.g., the ATG initiation codon and adjacent sequences. In cases where a coding sequence, its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases  
15 where only coding sequence (e.g., a mature protein coding sequence), or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon can be separately provided. The initiation codon is provided in the correct reading frame to facilitate transcription. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of  
20 enhancers appropriate to the cell system in use.

#### Expression Hosts

The present invention also relates to host cells which are transduced with vectors of the invention, and the production of polypeptides of the invention (including fragments thereof) by recombinant techniques. Host cells are genetically engineered (i.e, nucleic acids are introduced,  
25 e.g., transduced, transformed or transfected) with the vectors of this invention, which may be, for example, a cloning vector or an expression vector comprising the relevant nucleic acids herein. The vector is optionally a plasmid, a viral particle, a phage, a naked nucleic acids, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants, or amplifying the relevant gene. The culture  
30 conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to those skilled in the art and in the references cited herein, including, Sambrook and Ausubel.

The host cell can be a eukaryotic cell, such as a yeast cell, or a plant cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Plant protoplasts are also suitable for some

applications. For example, the DNA fragments are introduced into plant tissues, cultured plant cells or plant protoplasts by standard methods including electroporation (Fromm et al., (1985) Proc. Natl. Acad. Sci. USA 82, 5824, infection by viral vectors such as cauliflower mosaic virus (CaMV) (Hohn et al., (1982) Molecular Biology of Plant Tumors, (Academic Press, New York) pp. 549-560; US 4,407,956), high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface (Klein et al., (1987) Nature 327, 70-73), use of pollen as vector (WO 85/01856), or use of *Agrobacterium tumefaciens* or *A. rhizogenes* carrying a T-DNA plasmid in which DNA fragments are cloned. The T-DNA plasmid is transmitted to plant cells upon infection by *Agrobacterium tumefaciens*, and a portion is stably integrated into the plant genome (Horsch et al. (1984) Science 233:496-498; Fraley et al. (1983) Proc. Natl. Acad. Sci. USA 80, 4803).

The cell can include a nucleic acid of the invention which encodes a polypeptide, wherein the cells expresses a polypeptide of the invention. The cell can also include vector sequences, or the like. Furthermore, cells and transgenic plants which include any polypeptide or nucleic acid above or throughout this specification, e.g., produced by transduction of a vector of the invention, are an additional feature of the invention.

For long-term, high-yield production of recombinant proteins, stable expression can be used. Host cells transformed with a nucleotide sequence encoding a polypeptide of the invention are optionally cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The protein or fragment thereof produced by a recombinant cell may be secreted, membrane-bound, or contained intracellularly, depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides encoding mature proteins of the invention can be designed with signal sequences which direct secretion of the mature polypeptides through a prokaryotic or eukaryotic cell membrane.

#### Modified Amino Acids

Polypeptides of the invention may contain one or more modified amino acids. The presence of modified amino acids may be advantageous in, for example, increasing polypeptide half-life, reducing polypeptide antigenicity or toxicity, increasing polypeptide storage stability, or the like. Amino acid(s) are modified, for example, co-translationally or post-translationally during recombinant production or modified by synthetic or chemical means.

Non-limiting examples of a modified amino acid include incorporation or other use of acetylated amino acids, glycosylated amino acids, sulfated amino acids, prenylated (e.g., farnesylated, geranylgeranylated) amino acids, PEG modified (e.g., "PEGylated") amino acids,

biotinylated amino acids, carboxylated amino acids, phosphorylated amino acids, etc. References adequate to guide one of skill in the modification of amino acids are replete throughout the literature.

#### IDENTIFICATION OF ADDITIONAL FACTORS

5           A transcription factor provided by the present invention can also be used to identify additional endogenous or exogenous molecules that can affect a phenotype or trait of interest. On the one hand, such molecules include organic (small or large molecules) and/or inorganic compounds that affect expression of (i.e., regulate) a particular transcription factor. Alternatively, such molecules include endogenous molecules that are acted upon either at a transcriptional level by a transcription factor of the invention to modify a phenotype as desired. For example, the transcription factors can be employed to identify one or more downstream gene with which is subject to a regulatory effect of the transcription factor. In one approach, a transcription factor or transcription factor homologue of the invention is expressed in a host cell, e.g., a transgenic plant cell, tissue or explant, and expression products, either RNA or protein, of likely or random targets are monitored, e.g., by hybridization to a microarray of nucleic acid probes corresponding to genes expressed in a tissue or cell type of interest, by two-dimensional gel electrophoresis of protein products, or by any other method known in the art for assessing expression of gene products at the level of RNA or protein. Alternatively, a transcription factor of the invention can be used to identify promoter sequences (i.e., binding sites) involved in the regulation of a downstream target. After identifying a promoter sequence, interactions between the transcription factor and the promoter sequence can be modified by changing specific nucleotides in the promoter sequence or specific amino acids in the transcription factor that interact with the promoter sequence to alter a plant trait. Typically, transcription factor DNA binding sites are identified by gel shift assays. After identifying the promoter regions, the promoter region sequences can be employed in double-stranded DNA arrays to identify molecules that affect the interactions of the transcription factors with their promoters (Bulyk et al. (1999) Nature Biotechnology 17:573-577).

25           The identified transcription factors are also useful to identify proteins that modify the activity of the transcription factor. Such modification can occur by covalent modification, such as by phosphorylation, or by protein-protein (homo or-heteropolymer) interactions. Any method suitable for detecting protein-protein interactions can be employed. Among the methods that can be employed are co-immunoprecipitation, cross-linking and co-purification through gradients or chromatographic columns, and the two-hybrid yeast system.

The two-hybrid system detects protein interactions in vivo and is described in Chien, et al., (1991), Proc. Natl. Acad. Sci. USA 88, 9578-9582 and is commercially available from Clontech (Palo Alto, Calif.). In such a system, plasmids are constructed that encode two hybrid proteins: one consists of the DNA-binding domain of a transcription activator protein fused to the TF polypeptide and the other consists of the transcription activator protein's activation domain fused to an unknown protein that is encoded by a cDNA that has been recombined into the plasmid as part of a cDNA library. The DNA-binding domain fusion plasmid and the cDNA library are transformed into a strain of the yeast *Saccharomyces cerevisiae* that contains a reporter gene (e.g., lacZ) whose regulatory region contains the transcription activator's binding site. Either hybrid protein alone cannot activate transcription of the reporter gene. Interaction of the two hybrid proteins reconstitutes the functional activator protein and results in expression of the reporter gene, which is detected by an assay for the reporter gene product. Then, the library plasmids responsible for reporter gene expression are isolated and sequenced to identify the proteins encoded by the library plasmids. After identifying proteins that interact with the transcription factors, assays for compounds that interfere with the TF protein-protein interactions can be preformed.

#### IDENTIFICATION OF MODULATORS

In addition to the intracellular molecules described above, extracellular molecules that alter activity or expression of a transcription factor, either directly or indirectly, can be identified. For example, the methods can entail first placing a candidate molecule in contact with a plant or plant cell. The molecule can be introduced by topical administration, such as spraying or soaking of a plant, and then the molecule's effect on the expression or activity of the TF polypeptide or the expression of the polynucleotide monitored. Changes in the expression of the TF polypeptide can be monitored by use of polyclonal or monoclonal antibodies, gel electrophoresis or the like. Changes in the expression of the corresponding polynucleotide sequence can be detected by use of microarrays, Northern, quantitative PCR, or any other technique for monitoring changes in mRNA expression. These techniques are exemplified in Ausubel et al. (eds) Current Protocols in Molecular Biology, John Wiley & Sons (1998). Such changes in the expression levels can be correlated with modified plant traits and thus identified molecules can be useful for soaking or spraying on fruit, vegetable and grain crops to modify traits in plants.

Essentially any available composition can be tested for modulatory activity of expression or activity of any nucleic acid or polypeptide herein. Thus, available libraries of compounds such as chemicals, polypeptides, nucleic acids and the like can be tested for modulatory activity.

Often, potential modulator compounds can be dissolved in aqueous or organic (e.g., DMSO-based) solutions for easy delivery to the cell or plant of interest in which the activity of the modulator is to be tested. Optionally, the assays are designed to screen large modulator composition libraries by automating the assay steps and providing compounds from any  
5 convenient source to assays, which are typically run in parallel (e.g., in microtiter formats on microtiter plates in robotic assays).

In one embodiment, high throughput screening methods involve providing a combinatorial library containing a large number of potential compounds (potential modulator compounds). Such "combinatorial chemical libraries" are then screened in one or more assays, as  
10 described herein, to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as target compounds.

A combinatorial chemical library can be, e.g., a collection of diverse chemical compounds generated by chemical synthesis or biological synthesis. For example, a  
15 combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (e.g., in one example, amino acids) in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide compound of a set length). Exemplary libraries include peptide libraries, nucleic acid libraries, antibody libraries (see, e.g., Vaughn et al. (1996) Nature Biotechnology, 14(3):309-314 and PCT/US96/10287), carbohydrate  
20 libraries (see, e.g., Liang et al. Science (1996) 274:1520-1522 and U.S. Patent 5,593,853), peptide nucleic acid libraries (see, e.g., U.S. Patent 5,539,083), and small organic molecule libraries (see, e.g., benzodiazepines, Baum C&EN Jan 18, page 33 (1993); isoprenoids, U.S. Patent 5,569,588; thiazolidinones and metathiazanones, U.S. Patent 5,549,974; pyrrolidines, U.S. Patents 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent 5,506,337) and the like.

25 Preparation and screening of combinatorial or other libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (see, e.g., U.S. Patent 5,010,175, Furka, Int. J. Pept. Prot. Res. 37:487-493 (1991) and Houghton et al. Nature 354:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used.

30 In addition, as noted, compound screening equipment for high-throughput screening is generally available, e.g., using any of a number of well known robotic systems that have also been developed for solution phase chemistries useful in assay systems. These systems include automated workstations including an automated synthesis apparatus and robotic systems utilizing robotic arms. Any of the above devices are suitable for use with the present invention, e.g., for

high-throughput screening of potential modulators. The nature and implementation of modifications to these devices (if any) so that they can operate as discussed herein will be apparent to persons skilled in the relevant art.

5 Indeed, entire high throughput screening systems are commercially available. These systems typically automate entire procedures including all sample and reagent pipetting, liquid dispensing, timed incubations, and final readings of the microplate in detector(s) appropriate for the assay. These configurable systems provide high throughput and rapid start up as well as a high degree of flexibility and customization. Similarly, microfluidic implementations of screening are also commercially available.

10 The manufacturers of such systems provide detailed protocols the various high throughput. Thus, for example, Zymark Corp. provides technical bulletins describing screening systems for detecting the modulation of gene transcription, ligand binding, and the like. The integrated systems herein, in addition to providing for sequence alignment and, optionally, synthesis of relevant nucleic acids, can include such screening apparatus to identify modulators  
15 that have an effect on one or more polynucleotides or polypeptides according to the present invention.

In some assays it is desirable to have positive controls to ensure that the components of the assays are working properly. At least two types of positive controls are appropriate. That is, known transcriptional activators or inhibitors can be incubated with cells/plants/ etc. in one  
20 sample of the assay, and the resulting increase/decrease in transcription can be detected by measuring the resulting increase in RNA/ protein expression, etc., according to the methods herein. It will be appreciated that modulators can also be combined with transcriptional activators or inhibitors to find modulators which inhibit transcriptional activation or transcriptional repression. Either expression of the nucleic acids and proteins herein or any  
25 additional nucleic acids or proteins activated by the nucleic acids or proteins herein, or both, can be monitored.

In an embodiment, the invention provides a method for identifying compositions that modulate the activity or expression of a polynucleotide or polypeptide of the invention. For example, a test compound, whether a small or large molecule, is placed in contact with a cell,  
30 plant (or plant tissue or explant), or composition comprising the polynucleotide or polypeptide of interest and a resulting effect on the cell, plant, (or tissue or explant) or composition is evaluated by monitoring, either directly or indirectly, one or more of: expression level of the polynucleotide or polypeptide, activity (or modulation of the activity) of the polynucleotide or polypeptide. In some cases, an alteration in a plant phenotype can be detected following contact of a plant (or

plant cell, or tissue or explant) with the putative modulator, e.g., by modulation of expression or activity of a polynucleotide or polypeptide of the invention.

### SUBSEQUENCES

5 Also contemplated are uses of polynucleotides, also referred to herein as oligonucleotides, typically having at least 12 bases, preferably at least 15, more preferably at least 20, 30, or 50 bases, which hybridize under at least highly stringent (or ultra-high stringent or ultra-ultra- high stringent conditions) conditions to a polynucleotide sequence described above. The polynucleotides may be used as probes, primers, sense and antisense agents, and the like,  
10 according to methods as noted *supra*.

Subsequences of the polynucleotides of the invention, including polynucleotide fragments and oligonucleotides are useful as nucleic acid probes and primers. An oligonucleotide suitable for use as a probe or primer is at least about 15 nucleotides in length, more often at least about 18 nucleotides, often at least about 21 nucleotides, frequently at least about 30 nucleotides,  
15 or about 40 nucleotides, or more in length. A nucleic acid probe is useful in hybridization protocols, e.g., to identify additional polypeptide homologues of the invention, including protocols for microarray experiments. Primers can be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then extended along the target DNA strand by a DNA polymerase enzyme. Primer  
20 pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods. See Sambrook and Ausubel, *supra*.

In addition, the invention includes an isolated or recombinant polypeptide including a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotides of the invention. For example, such polypeptides, or domains or fragments  
25 thereof, can be used as immunogens, e.g., to produce antibodies specific for the polypeptide sequence, or as probes for detecting a sequence of interest. A subsequence can range in size from about 15 amino acids in length up to and including the full length of the polypeptide.

### PRODUCTION OF TRANSGENIC PLANTS

#### Modification of Traits

30 The polynucleotides of the invention are favorably employed to produce transgenic plants with various traits, or characteristics, that have been modified in a desirable manner, e.g., to improve the seed characteristics of a plant. For example, alteration of expression levels or patterns (e.g., spatial or temporal expression patterns) of one or more of the transcription factors

(or transcription factor homologues) of the invention, as compared with the levels of the same protein found in a wild type plant, can be used to modify a plant's traits. An illustrative example of trait modification, improved biochemical characteristics, by altering expression levels of a particular transcription factor is described further in the Examples and the Sequence Listing.

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#### Antisense and Cosuppression Approaches

In addition to expression of the nucleic acids of the invention as gene replacement or plant phenotype modification nucleic acids, the nucleic acids are also useful for sense and anti-sense suppression of expression, e.g., to down-regulate expression of a nucleic acid of the invention, e.g., as a further mechanism for modulating plant phenotype. That is, the nucleic acids of the invention, or subsequences or anti-sense sequences thereof, can be used to block expression of naturally occurring homologous nucleic acids. A variety of sense and anti-sense technologies are known in the art, e.g., as set forth in Lichtenstein and Nellen (1997) Antisense Technology: A Practical Approach IRL Press at Oxford University, Oxford, England. In general, sense or anti-sense sequences are introduced into a cell, where they are optionally amplified, e.g., by transcription. Such sequences include both simple oligonucleotide sequences and catalytic sequences such as ribozymes.

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For example, a reduction or elimination of expression (i.e., a "knock-out") of a transcription factor or transcription factor homologue polypeptide in a transgenic plant, e.g., to modify a plant trait, can be obtained by introducing an antisense construct corresponding to the polypeptide of interest as a cDNA. For antisense suppression, the transcription factor or homologue cDNA is arranged in reverse orientation (with respect to the coding sequence) relative to the promoter sequence in the expression vector. The introduced sequence need not be the full length cDNA or gene, and need not be identical to the cDNA or gene found in the plant type to be transformed. Typically, the antisense sequence need only be capable of hybridizing to the target gene or RNA of interest. Thus, where the introduced sequence is of shorter length, a higher degree of homology to the endogenous transcription factor sequence will be needed for effective antisense suppression. While antisense sequences of various lengths can be utilized, preferably, the introduced antisense sequence in the vector will be at least 30 nucleotides in length, and improved antisense suppression will typically be observed as the length of the antisense sequence increases. Preferably, the length of the antisense sequence in the vector will be greater than 100 nucleotides. Transcription of an antisense construct as described results in the production of RNA molecules that are the reverse complement of mRNA molecules transcribed from the endogenous transcription factor gene in the plant cell.

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Suppression of endogenous transcription factor gene expression can also be achieved using a ribozyme. Ribozymes are RNA molecules that possess highly specific endoribonuclease activity. The production and use of ribozymes are disclosed in U.S. Patent No. 4,987,071 and U.S. Patent No. 5,543,508. Synthetic ribozyme sequences including antisense RNAs can be used to confer RNA cleaving activity on the antisense RNA, such that endogenous mRNA molecules that hybridize to the antisense RNA are cleaved, which in turn leads to an enhanced antisense inhibition of endogenous gene expression.

Vectors in which RNA encoded by a transcription factor or transcription factor homologue cDNA is over-expressed can also be used to obtain co-suppression of a corresponding endogenous gene, e.g., in the manner described in U.S. Patent No. 5,231,020 to Jorgensen. Such co-suppression (also termed sense suppression) does not require that the entire transcription factor cDNA be introduced into the plant cells, nor does it require that the introduced sequence be exactly identical to the endogenous transcription factor gene of interest. However, as with antisense suppression, the suppressive efficiency will be enhanced as specificity of hybridization is increased, e.g., as the introduced sequence is lengthened, and/or as the sequence similarity between the introduced sequence and the endogenous transcription factor gene is increased.

Vectors expressing an untranslatable form of the transcription factor mRNA, e.g., sequences comprising one or more stop codon, or nonsense mutation) can also be used to suppress expression of an endogenous transcription factor, thereby reducing or eliminating its activity and modifying one or more traits. Methods for producing such constructs are described in U.S. Patent No. 5,583,021. Preferably, such constructs are made by introducing a premature stop codon into the transcription factor gene. Alternatively, a plant trait can be modified by gene silencing using double-strand RNA (Sharp (1999) Genes and Development 13: 139-141).

Another method for abolishing the expression of a gene is by insertion mutagenesis using the T-DNA of *Agrobacterium tumefaciens*. After generating the insertion mutants, the mutants can be screened to identify those containing the insertion in a transcription factor or transcription factor homologue gene. Plants containing a single transgene insertion event at the desired gene can be crossed to generate homozygous plants for the mutation (Koncz et al. (1992) Methods in Arabidopsis Research, World Scientific).

Alternatively, a plant phenotype can be altered by eliminating an endogenous gene, such as a transcription factor or transcription factor homologue, e.g., by homologous recombination (Kempin et al. (1997) Nature 389:802).

A plant trait can also be modified by using the cre-lox system (for example, as described in US Pat. No. 5,658,772). A plant genome can be modified to include first and second lox sites

that are then contacted with a Cre recombinase. If the lox sites are in the same orientation, the intervening DNA sequence between the two sites is excised. If the lox sites are in the opposite orientation, the intervening sequence is inverted.

5 The polynucleotides and polypeptides of this invention can also be expressed in a plant in the absence of an expression cassette by manipulating the activity or expression level of the endogenous gene by other means. For example, by ectopically expressing a gene by T-DNA activation tagging (Ichikawa et al. (1997) Nature 390 698-701; Kakimoto et al. (1996) Science 274: 982-985). This method entails transforming a plant with a gene tag containing multiple transcriptional enhancers and once the tag has inserted into the genome, expression of a flanking  
10 gene coding sequence becomes deregulated. In another example, the transcriptional machinery in a plant can be modified so as to increase transcription levels of a polynucleotide of the invention (See, e.g., PCT Publications WO 96/06166 and WO 98/53057 which describe the modification of the DNA binding specificity of zinc finger proteins by changing particular amino acids in the DNA binding motif).

15 The transgenic plant can also include the machinery necessary for expressing or altering the activity of a polypeptide encoded by an endogenous gene, for example by altering the phosphorylation state of the polypeptide to maintain it in an activated state.

Transgenic plants (or plant cells, or plant explants, or plant tissues) incorporating the polynucleotides of the invention and/or expressing the polypeptides of the invention can be  
20 produced by a variety of well established techniques as described above. Following construction of a vector, most typically an expression cassette, including a polynucleotide, e.g., encoding a transcription factor or transcription factor homologue, of the invention, standard techniques can be used to introduce the polynucleotide into a plant, a plant cell, a plant explant or a plant tissue of interest. Optionally, the plant cell, explant or tissue can be regenerated to produce a transgenic  
25 plant.

The plant can be any higher plant, including gymnosperms, monocotyledonous and dicotyledonous plants. Suitable protocols are available for *Leguminosae* (alfalfa, soybean, clover, etc.), *Umbelliferae* (carrot, celery, parsnip), *Cruciferae* (cabbage, radish, rapeseed, broccoli, etc.), *Curcubitaceae* (melons and cucumber), *Gramineae* (wheat, corn, rice, barley, millet, etc.),  
30 *Solanaceae* (potato, tomato, tobacco, peppers, etc.), and various other crops. See protocols described in Ammirato et al. (1984) Handbook of Plant Cell Culture -Crop Species. Macmillan Publ. Co. Shimamoto et al. (1989) Nature 338:274-276; Fromm et al. (1990) Bio/Technology 8:833-839; and Vasil et al. (1990) Bio/Technology 8:429-434.

Transformation and regeneration of both monocotyledonous and dicotyledonous plant cells is now routine, and the selection of the most appropriate transformation technique will be determined by the practitioner. The choice of method will vary with the type of plant to be transformed; those skilled in the art will recognize the suitability of particular methods for given plant types. Suitable methods can include, but are not limited to: electroporation of plant protoplasts; liposome-mediated transformation; polyethylene glycol (PEG) mediated transformation; transformation using viruses; micro-injection of plant cells; micro-projectile bombardment of plant cells; vacuum infiltration; and *Agrobacterium tumefaciens* mediated transformation. Transformation means introducing a nucleotide sequence in a plant in a manner to cause stable or transient expression of the sequence.

Successful examples of the modification of plant characteristics by transformation with cloned sequences which serve to illustrate the current knowledge in this field of technology, and which are herein incorporated by reference, include: U.S. Patent Nos. 5,571,706; 5,677,175; 5,510,471; 5,750,386; 5,597,945; 5,589,615; 5,750,871; 5,268,526; 5,780,708; 5,538,880; 5,773,269; 5,736,369 and 5,610,042.

Following transformation, plants are preferably selected using a dominant selectable marker incorporated into the transformation vector. Typically, such a marker will confer antibiotic or herbicide resistance on the transformed plants, and selection of transformants can be accomplished by exposing the plants to appropriate concentrations of the antibiotic or herbicide.

After transformed plants are selected and grown to maturity, those plants showing a modified trait are identified. The modified trait can be any of those traits described above. Additionally, to confirm that the modified trait is due to changes in expression levels or activity of the polypeptide or polynucleotide of the invention can be determined by analyzing mRNA expression using Northern blots, RT-PCR or microarrays, or protein expression using immunoblots or Western blots or gel shift assays.

#### INTEGRATED SYSTEMS—SEQUENCE IDENTITY

Additionally, the present invention may be an integrated system, computer or computer readable medium that comprises an instruction set for determining the identity of one or more sequences in a database. In addition, the instruction set can be used to generate or identify sequences that meet any specified criteria. Furthermore, the instruction set may be used to associate or link certain functional benefits, such improved biochemical characteristics, with one or more identified sequence.

For example, the instruction set can include, e.g., a sequence comparison or other alignment program, e.g., an available program such as, for example, the Wisconsin Package Version 10.0, such as BLAST, FASTA, PILEUP, FINDPATTERNS or the like (GCG, Madison, WI). Public sequence databases such as GenBank, EMBL, Swiss-Prot and PIR or private  
5 sequence databases such as PhytoSeq (Incyte Pharmaceuticals, Palo Alto, CA) can be searched.

Alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. U.S.A. 85: 2444, by computerized  
10 implementations of these algorithms. After alignment, sequence comparisons between two (or more) polynucleotides or polypeptides are typically performed by comparing sequences of the two sequences over a comparison window to identify and compare local regions of sequence similarity. The comparison window can be a segment of at least about 20 contiguous positions, usually about 50 to about 200, more usually about 100 to about 150 contiguous positions. A  
15 description of the method is provided in Ausubel et al., *supra*.

A variety of methods of determining sequence relationships can be used, including manual alignment and computer assisted sequence alignment and analysis. This later approach is a preferred approach in the present invention, due to the increased throughput afforded by computer assisted methods. As noted above, a variety of computer programs for performing  
20 sequence alignment are available, or can be produced by one of skill.

One example algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al. J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available, e.g., through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This  
25 algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al., *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them.  
30 The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each

direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment.

- 5 The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915).

- 10 In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see*, e.g., Karlin & Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For  
15 example, a nucleic acid is considered similar to a reference sequence (and, therefore, in this context, homologous) if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, or less than about 0.01, and or even less than about 0.001. An additional example of a useful sequence alignment algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using  
20 progressive, pairwise alignments. The program can align, e.g., up to 300 sequences of a maximum length of 5,000 letters.

- The integrated system, or computer typically includes a user input interface allowing a user to selectively view one or more sequence records corresponding to the one or more character strings, as well as an instruction set which aligns the one or more character strings with each other  
25 or with an additional character string to identify one or more region of sequence similarity. The system may include a link of one or more character strings with a particular phenotype or gene function. Typically, the system includes a user readable output element which displays an alignment produced by the alignment instruction set.

- The methods of this invention can be implemented in a localized or distributed  
30 computing environment. In a distributed environment, the methods may implemented on a single computer comprising multiple processors or on a multiplicity of computers. The computers can be linked, e.g. through a common bus, but more preferably the computer(s) are nodes on a network. The network can be a generalized or a dedicated local or wide-area network and, in certain preferred embodiments, the computers may be components of an intra-net or an internet.

Thus, the invention provides methods for identifying a sequence similar or homologous to one or more polynucleotides as noted herein, or one or more target polypeptides encoded by the polynucleotides, or otherwise noted herein and may include linking or associating a given plant phenotype or gene function with a sequence. In the methods, a sequence database is  
5 provided (locally or across an inter or intra net) and a query is made against the sequence database using the relevant sequences herein and associated plant phenotypes or gene functions.

Any sequence herein can be entered into the database, before or after querying the database. This provides for both expansion of the database and, if done before the querying step, for insertion of control sequences into the database. The control sequences can be detected by the  
10 query to ensure the general integrity of both the database and the query. As noted, the query can be performed using a web browser based interface. For example, the database can be a centralized public database such as those noted herein, and the querying can be done from a remote terminal or computer across an internet or intranet.

### EXAMPLES

15 The following examples are intended to illustrate but not limit the present invention.

#### EXAMPLE I. FULL LENGTH GENE IDENTIFICATION AND CLONING

Putative transcription factor sequences (genomic or ESTs) related to known transcription factors were identified in the *Arabidopsis thaliana* GenBank database using the tblastn sequence analysis program using default parameters and a P-value cutoff threshold of -4 or -5 or lower,  
20 depending on the length of the query sequence. Putative transcription factor sequence hits were then screened to identify those containing particular sequence strings. If the sequence hits contained such sequence strings, the sequences were confirmed as transcription factors.

Alternatively, *Arabidopsis thaliana* cDNA libraries derived from different tissues or treatments, or genomic libraries were screened to identify novel members of a transcription  
25 family using a low stringency hybridization approach. Probes were synthesized using gene specific primers in a standard PCR reaction (annealing temperature 60° C) and labeled with <sup>32</sup>P dCTP using the High Prime DNA Labeling Kit (Boehringer Mannheim). Purified radiolabelled probes were added to filters immersed in Church hybridization medium (0.5 M NaPO<sub>4</sub> pH 7.0, 7% SDS, 1 % w/v bovine serum albumin) and hybridized overnight at 60 °C with shaking. Filters  
30 were washed two times for 45 to 60 minutes with 1xSCC, 1% SDS at 60° C.

To identify additional sequence 5' or 3' of a partial cDNA sequence in a cDNA library, 5' and 3' rapid amplification of cDNA ends (RACE) was performed using the Marathon™ cDNA amplification kit (Clontech, Palo Alto, CA). Generally, the method entailed first isolating

poly(A) mRNA, performing first and second strand cDNA synthesis to generate double stranded cDNA, blunting cDNA ends, followed by ligation of the Marathon™ Adaptor to the cDNA to form a library of adaptor-ligated ds cDNA.

Gene-specific primers were designed to be used along with adaptor specific primers for both 5' and 3' RACE reactions. Nested primers, rather than single primers, were used to increase PCR specificity. Using 5' and 3' RACE reactions, 5' and 3' RACE fragments were obtained, sequenced and cloned. The process can be repeated until 5' and 3' ends of the full-length gene were identified. Then the full-length cDNA was generated by PCR using primers specific to 5' and 3' ends of the gene by end-to-end PCR.

#### 10        EXAMPLE II. CONSTRUCTION OF EXPRESSION VECTORS

The sequence was amplified from a genomic or cDNA library using primers specific to sequences upstream and downstream of the coding region. The expression vector was pMEN20 or pMEN65, which are both derived from pMON316 (Sanders et al, (1987) Nucleic Acids Research 15:1543-58) and contain the CaMV 35S promoter to express transgenes. To clone the sequence into the vector, both pMEN20 and the amplified DNA fragment were digested separately with SalI and NotI restriction enzymes at 37° C for 2 hours. The digestion products were subject to electrophoresis in a 0.8% agarose gel and visualized by ethidium bromide staining. The DNA fragments containing the sequence and the linearized plasmid were excised and purified by using a Qiaquick gel extraction kit (Qiagen, CA). The fragments of interest were ligated at a ratio of 3:1 (vector to insert). Ligation reactions using T4 DNA ligase (New England Biolabs, MA) were carried out at 16° C for 16 hours. The ligated DNAs were transformed into competent cells of the *E. coli* strain DH5alpha by using the heat shock method. The transformations were plated on LB plates containing 50 mg/l kanamycin (Sigma).

Individual colonies were grown overnight in five milliliters of LB broth containing 50 mg/l kanamycin at 37° C. Plasmid DNA was purified by using Qiaquick Mini Prep kits (Qiagen, CA).

#### 25        EXAMPLE III. TRANSFORMATION OF AGROBACTERIUM WITH THE EXPRESSION VECTOR

After the plasmid vector containing the gene was constructed, the vector was used to transform *Agrobacterium tumefaciens* cells expressing the gene products. The stock of *Agrobacterium tumefaciens* cells for transformation were made as described by Nagel et al. (1990) FEMS Microbiol Letts. 67: 325-328. *Agrobacterium* strain ABI was grown in 250 ml LB medium (Sigma) overnight at 28°C with shaking until an absorbance ( $A_{600}$ ) of 0.5 – 1.0 was

reached. Cells were harvested by centrifugation at 4,000 x g for 15 min at 4° C. Cells were then resuspended in 250 µl chilled buffer (1 mM HEPES, pH adjusted to 7.0 with KOH). Cells were centrifuged again as described above and resuspended in 125 µl chilled buffer. Cells were then centrifuged and resuspended two more times in the same HEPES buffer as described above at a volume of 100 µl and 750 µl, respectively. Resuspended cells were then distributed into 40 µl aliquots, quickly frozen in liquid nitrogen, and stored at -80° C.

*Agrobacterium* cells were transformed with plasmids prepared as described above following the protocol described by Nagel et al. For each DNA construct to be transformed, 50 – 100 ng DNA (generally resuspended in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) was mixed with 40 µl of *Agrobacterium* cells. The DNA/cell mixture was then transferred to a chilled cuvette with a 2mm electrode gap and subject to a 2.5 kV charge dissipated at 25 µF and 200 µF using a Gene Pulser II apparatus (Bio-Rad). After electroporation, cells were immediately resuspended in 1.0 ml LB and allowed to recover without antibiotic selection for 2 – 4 hours at 28° C in a shaking incubator. After recovery, cells were plated onto selective medium of LB broth containing 100 µg/ml spectinomycin (Sigma) and incubated for 24-48 hours at 28° C. Single colonies were then picked and inoculated in fresh medium. The presence of the plasmid construct was verified by PCR amplification and sequence analysis.

#### EXAMPLE IV. TRANSFORMATION OF *ARABIDOPSIS* PLANTS WITH *AGROBACTERIUM TUMEFACIENS* WITH EXPRESSION VECTOR

After transformation of *Agrobacterium tumefaciens* with plasmid vectors containing the gene, single *Agrobacterium* colonies were identified, propagated, and used to transform *Arabidopsis* plants. Briefly, 500 ml cultures of LB medium containing 50 mg/l kanamycin were inoculated with the colonies and grown at 28° C with shaking for 2 days until an absorbance ( $A_{600}$ ) of > 2.0 is reached. Cells were then harvested by centrifugation at 4,000 x g for 10 min, and resuspended in infiltration medium (1/2 X Murashige and Skoog salts (Sigma), 1 X Gamborg's B-5 vitamins (Sigma), 5.0% (w/v) sucrose (Sigma), 0.044 µM benzylamino purine (Sigma), 200 µl/L Silwet L-77 (Lehle Seeds) until an absorbance ( $A_{600}$ ) of 0.8 was reached.

Prior to transformation, *Arabidopsis thaliana* seeds (ecotype Columbia) were sown at a density of ~10 plants per 4" pot onto Pro-Mix BX potting medium (Hummert International) covered with fiberglass mesh (18 mm X 16 mm). Plants were grown under continuous illumination (50-75 µE/m<sup>2</sup>/sec) at 22-23° C with 65-70% relative humidity. After about 4 weeks, primary inflorescence stems (bolts) are cut off to encourage growth of multiple secondary bolts. After flowering of the mature secondary bolts, plants were prepared for transformation by removal of all siliques and opened flowers.



The pots were then immersed upside down in the mixture of *Agrobacterium* infiltration medium as described above for 30 sec, and placed on their sides to allow draining into a 1' x 2' flat surface covered with plastic wrap. After 24 h, the plastic wrap was removed and pots are turned upright. The immersion procedure was repeated one week later, for a total of two  
5 immersions per pot. Seeds were then collected from each transformation pot and analyzed following the protocol described below.

**EXAMPLE V. IDENTIFICATION OF ARABIDOPSIS PRIMARY TRANSFORMANTS**

Seeds collected from the transformation pots were sterilized essentially as follows. Seeds  
10 were dispersed into in a solution containing 0.1% (v/v) Triton X-100 (Sigma) and sterile H<sub>2</sub>O and washed by shaking the suspension for 20 min. The wash solution was then drained and replaced with fresh wash solution to wash the seeds for 20 min with shaking. After removal of the second wash solution, a solution containing 0.1% (v/v) Triton X-100 and 70% ethanol (Equistar) was added to the seeds and the suspension was shaken for 5 min. After removal of the  
15 ethanol/detergent solution, a solution containing 0.1% (v/v) Triton X-100 and 30% (v/v) bleach (Clorox) was added to the seeds, and the suspension was shaken for 10 min. After removal of the bleach/detergent solution, seeds were then washed five times in sterile distilled H<sub>2</sub>O. The seeds were stored in the last wash water at 4°C for 2 days in the dark before being plated onto antibiotic selection medium (1 X Murashige and Skoog salts (pH adjusted to 5.7 with 1M KOH), 1 X  
20 Gamborg's B-5 vitamins, 0.9% phytagar (Life Technologies), and 50 mg/l kanamycin). Seeds were germinated under continuous illumination (50-75  $\mu\text{E}/\text{m}^2/\text{sec}$ ) at 22-23°C. After 7-10 days of growth under these conditions, kanamycin resistant primary transformants (T<sub>1</sub> generation) were visible and obtained. These seedlings were transferred first to fresh selection plates where the seedlings continued to grow for 3-5 more days, and then to soil (Pro-Mix BX potting  
25 medium).

Primary transformants were crossed and progeny seeds (T<sub>2</sub>) collected; kanamycin resistant seedlings were selected and analyzed. The expression levels of the recombinant polynucleotides in the transformants varies from about a 5% expression level increase to a least a 100% expression level increase. Similar observations are made with respect to polypeptide level  
30 expression.

EXAMPLE VI. IDENTIFICATION OF ARABIDOPSIS PLANTS WITH  
TRANSCRIPTION FACTOR GENE KNOCKOUTS

The screening of insertion mutagenized *Arabidopsis* collections for null mutants in a known target gene was essentially as described in Krysan et al (1999) Plant Cell 11:2283-2290. Briefly, gene-specific primers, nested by 5-250 base pairs to each other, were designed from the 5' and 3' regions of a known target gene. Similarly, nested sets of primers were also created specific to each of the T-DNA or transposon ends (the "right" and "left" borders). All possible combinations of gene specific and T-DNA/transposon primers were used to detect by PCR an insertion event within or close to the target gene. The amplified DNA fragments were then sequenced which allows the precise determination of the T-DNA/transposon insertion point relative to the target gene. Insertion events within the coding or intervening sequence of the genes were deconvoluted from a pool comprising a plurality of insertion events to a single unique mutant plant for functional characterization. The method is described in more detail in Yu and Adam, US Application Serial No. 09/177,733 filed October 23, 1998.

EXAMPLE VII. IDENTIFICATION OF MODIFIED BIOCHEMICAL  
CHARACTERISTICS PHENOTYPE IN OVEREXPRESSOR OR GENE KNOCKOUT  
PLANTS

Experiments were performed to identify those transformants or knockouts that exhibited modified biochemical characteristics. Among the biochemicals that were assayed were insoluble sugars, such as arabinose, fucose, galactose, mannose, rhamnose or xylose or the like; prenol lipids, such as lutein, beta-carotene, xanthophyll-1, xanthophyll-2, chlorophylls A or B, or alpha-, delta- or gamma-tocopherol or the like; fatty acids, such as 16:0 (palmitic acid), 16:1 (palmitoleic acid), 18:0 (stearic acid), 18:1 (oleic acid), 18:2 (linoleic acid), 20:0, 18:3 (linolenic acid), 20:1 (eicosenoic acid), 20:2, 22:1 (erucic acid) or the like; waxes, such as by altering the levels of C29, C31, or C33 alkanes; sterols, such as brassicasterol, campesterol, stigmasterol, sitosterol or stigmasterol or the like, glucosinolates, protein or oil levels

Fatty acids were measured using two methods depending on whether the tissue was from leaves or seeds. For leaves, lipids were extracted and esterified with hot methanolic H<sub>2</sub>SO<sub>4</sub> and partitioned into hexane from methanolic brine. For seed fatty acids, seeds were pulverized and extracted in methanol:heptane:toluene:2,2-dimethoxypropane:H<sub>2</sub>SO<sub>4</sub> (39:34:20:5:2) for 90 minutes at 80°C. After cooling to room temperature the upper phase, containing the seed fatty acid esters, was subjected to GC analysis. Fatty acid esters from both seed and leaf tissues were analyzed with a Supelco SP-2330 column.

Glucosinolates were purified from seeds or leaves by first heating the tissue at 95°C for 10 minutes. Preheated ethanol:water (50:50) is and after heating at 95°C for a further 10 minutes, the extraction solvent is applied to a DEAE Sephadex column which had been previously equilibrated with 0.5 M pyridine acetate. Desulfoglucosinolates were eluted with 300 ul water and analyzed by reverse phase HPLC monitoring at 226 nm.

For wax alkanes, samples were extracted using an identical method as fatty acids and extracts were analyzed on a HP 5890 GC coupled with a 5973 MSD. Samples were chromatographed on a J&W DB35 mass spectrometer (J&W Scientific).

To measure prenyl lipids levels, seeds or leaves were pulverized with 1 to 2% pyrogallol as an antioxidant. For seeds, extracted samples were filtered and a portion removed for tocopherol and carotenoid/chlorophyll analysis by HPLC. The remaining material was saponified for sterol determination. For leaves, an aliquot was removed and diluted with methanol and chlorophyll A, chlorophyll B, and total carotenoids measured by spectrophotometry by determining absorbance at 665.2 nm, 652.5 nm, and 470 nm. An aliquot was removed for tocopherol and carotenoid/chlorophyll composition by HPLC using a Waters uBondapak C18 column (4.6 mm x 150 mm). The remaining methanolic solution was saponified with 10% KOH at 80°C for one hour. The samples were cooled and diluted with a mixture of methanol and water. A solution of 2% methylene chloride in hexane was mixed in and the samples were centrifuged. The aqueous methanol phase was again re-extracted 2% methylene chloride in hexane and, after centrifugation, the two upper phases were combined and evaporated. 2% methylene chloride in hexane was added to the tubes and the samples were then extracted with one ml of water. The upper phase was removed, dried, and resuspended in 400 ul of 2% methylene chloride in hexane and analyzed by gas chromatography using a 50 m DB-5ms (0.25 mm ID, 0.25 um phase, J&W Scientific).

Insoluble sugar levels were measured by the method essentially described by Reiter et al., Plant Journal 12:335-345. This method analyzes the neutral sugar composition of cell wall polymers found in *Arabidopsis* leaves. Soluble sugars were separated from sugar polymers by extracting leaves with hot 70% ethanol. The remaining residue containing the insoluble polysaccharides was then acid hydrolyzed with allose added as an internal standard. Sugar monomers generated by the hydrolysis were then reduced to the corresponding alditols by treatment with NaBH<sub>4</sub>, then were acetylated to generate the volatile alditol acetates which were then analyzed by GC-FID. Identity of the peaks was determined by comparing the retention times of known sugars converted to the corresponding alditol acetates with the retention times of peaks from wild-type plant extracts. Alditol acetates were analyzed on a Supelco SP-2330 capillary

column (30 m x 250  $\mu$ m x 0.2  $\mu$ m) using a temperature program beginning at 180° C for 2 minutes followed by an increase to 220° C in 4 minutes. After holding at 220° C for 10 minutes, the oven temperature is increased to 240° C in 2 minutes and held at this temperature for 10 minutes and brought back to room temperature.

- 5 To identify plants with alterations in total seed oil or protein content, 150mg of seeds from T2 progeny plants were subjected to analysis by Near Infrared Reflectance (NIR) using a Foss NirSystems Model 6500 with a spinning cup transport system.

- 10 Table 3 shows the phenotypes observed for particular overexpressor or knockout plants and provides the SEQ ID No., the internal reference code (GID), whether a knockout or overexpressor plant was analyzed and the observed phenotype.

**Table 3**

SEQ ID No.	GID	Knockout (KO) or overexpressor (OE)	Phenotype observed
1	G214	OE	Increase in leaf fatty acids, for example 100% increase in 18:0 fatty acid. Also up to 100% increase in leaf chlorophyll and 100% increase in leaf carotenoids
3	G231	OE	Up to 5% increase in leaf 18:3 fatty acid
5	G274	OE	Up to 50% increase in leaf arabinose
7	G307	OE	Altered in leaf insoluble sugars, for example up to 44% decrease in mannose.
9	G346	OE	Altered leaf fatty acids, for example 25% increase in 16:3 and altered insoluble sugars, for example up to 25% increase in fucose
11	G598	OE	Altered in insoluble sugars, for example up to 20% decrease in rhamnose and up to 10% increase in galactose
13	G605	OE	Altered in leaf fatty acids, for example up to 20% increase in 16:1 fatty acid.
15	G777	OE	Altered in insoluble sugars, for example up to 60% increase in leaf rhamnose
17	G869	OE	Alteration in leaf fatty acids eg up to 39% decrease in 16:0 fatty acid; up to 43% increase in fucose
19	G1133	OE	Up to 34% decrease in leaf lutein
21	G1266	OE	Alteration in leaf fatty acids, for example up to 50% increase in 18:0 fatty acid. Alterations in leaf insoluble sugars, for example a 45% decrease in rhamnose
23	G1324	OE	Up to 65% decrease in leaf lutein and up to 84% increase in leaf xanthophyll

25	G1337	OE	Alteration in leaf fatty acids, for example up to 28% increase in 18:1 fatty acid
27	G975	OE	Up to 13-fold increase in wax in leaves

For a particular overexpressor that shows a less beneficial biochemical characteristic, it may be more useful to select a plant with a decreased expression of the particular transcription factor. For a particular knockout that shows a less beneficial biochemical characteristic, it may be more useful to select a plant with an increased expression of the particular transcription factor.

#### EXAMPLE VIII. IDENTIFICATION OF HOMOLOGOUS SEQUENCES

Homologous sequences from *Arabidopsis* and plant species other than *Arabidopsis* were identified using database sequence search tools, such as the Basic Local Alignment Search Tool (BLAST) (Altschul et al. (1990) *J. Mol. Biol.* 215:403-410; and Altschul et al. (1997) *Nucl. Acid Res.* 25: 3389-3402). The tblastx sequence analysis programs were employed using the BLOSUM-62 scoring matrix (Henikoff, S. and Henikoff, J. G. (1992) *Proc. Natl. Acad. Sci. USA* 89: 10915-10919).

Identified *Arabidopsis* homologous sequences are provided in Figure 2 and included in the Sequence Listing. The percent sequence identity among these sequences is as low as 47% sequence identity. Additionally, the entire NCBI GenBank database was filtered for sequences from all plants except *Arabidopsis thaliana* by selecting all entries in the NCBI GenBank database associated with NCBI taxonomic ID 33090 (Viridiplantae; all plants) and excluding entries associated with taxonomic ID 3701 (*Arabidopsis thaliana*). These sequences were compared to sequences representing genes of SEQ IDs Nos. 1-54 on 9/26/2000 using the Washington University TBLASTX algorithm (version 2.0a19MP). For each gene of SEQ IDs Nos. 1-54, individual comparisons were ordered by probability score (P-value), where the score reflects the probability that a particular alignment occurred by chance. For example, a score of  $3.6e-40$  is  $3.6 \times 10^{-40}$ . For up to ten species, the gene with the lowest P-value (and therefore the most likely homolog) is listed in Figure 3.

In addition to P-values, comparisons were also scored by percentage identity. Percentage identity reflects the degree to which two segments of DNA or protein are identical over a particular length. The ranges of percent identity between the non-*Arabidopsis* genes shown in Figure 3 and the *Arabidopsis* genes in the sequence listing are: SEQ ID No. 1: 38%-89%; SEQ ID No. 3: 64%-88%; SEQ ID No. 5: 44%-84%; SEQ ID No. 7: 35%-86%; SEQ ID No. 9: 43%-77%; SEQ ID No. 11: 43%-85%; SEQ ID No. 13: 41%-76%; SEQ ID No. 15: 34%-63%; SEQ ID No. 17: 31%-68%; SEQ ID No. 19: 26%-44%; SEQ ID No. 21: 52%-70%; SEQ ID No. 23: 37%-

93%; SEQ ID No. 25: 37%-58%; SEQ ID No. 27: 48%-92%; SEQ ID No. 29: 42%-88%; SEQ ID No. 31: 47%-90%; SEQ ID No. 33: 45%-69%; SEQ ID No. 35: 42%-94%; SEQ ID No. 37: 38%-85%; SEQ ID No. 39: 49%-93%; SEQ ID No. 41: 36%-64%; and SEQ ID No. 43: 36%-70%.

5       The polynucleotides and polypeptides in the Sequence Listing and the identified homologous sequences may be stored in a computer system and have associated or linked with the sequences a function, such as that the polynucleotides and polypeptides are useful for modifying the biochemical characteristics of a plant.

10       All references, publications, patents and other documents herein are incorporated by reference in their entirety for all purposes. Although the invention has been described with reference to the embodiments and examples above, it should be understood that various modifications can be made without departing from the spirit of the invention.

What is claimed is:

1. A transgenic plant with a modified biochemical characteristic, which plant comprises a recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of:
  - 5 (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-22, or a complementary nucleotide sequence thereof;
  - (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a);
  - (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-10 1, where N=1-22, or a complementary nucleotide sequence thereof;
  - (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c);
  - (e) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of one or more of: (a), (b), (c), or (d);
  - (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of 15 any of (a)-(e);
  - (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide that modifies a plant's biochemical characteristic;
  - (h) a nucleotide sequence having at least 31% sequence identity to a nucleotide sequence 20 of any of (a)-(g);
  - (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g);
  - (j) a nucleotide sequence which encodes a polypeptide having at least 31% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-22;
  - 25 (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-22; and
  - (l) a nucleotide sequence which encodes a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-22.
- 30 2. The transgenic plant of claim 1, further comprising a constitutive, inducible, or tissue-active promoter operably linked to said nucleotide sequence.
3. The transgenic plant of claim 1, wherein the plant is selected from the group consisting of: soybean, wheat, corn, potato, cotton, rice, oilseed rape, sunflower, alfalfa, sugarcane, turf,

banana, blackberry, blueberry, strawberry, raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits, and vegetable brassicas.

5

4. An isolated or recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-22, or a complementary nucleotide sequence thereof;
- 10 (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a);
- (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-22, or a complementary nucleotide sequence thereof;
- (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c);
- 15 (e) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of one or more of: (a), (b), (c), or (d);
- (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e);
- (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which  
20 subsequence or fragment encodes a polypeptide that modifies a plant's biochemical characteristic;
- (h) a nucleotide sequence having at least 31% sequence identity to a nucleotide sequence of any of (a)-(g);
- (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide  
25 sequence of any of (a)-(g);
- (j) a nucleotide sequence which encodes a polypeptide having at least 31% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-22;
- (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-22; and
- 30 (l) a nucleotide sequence which encodes a conserved domain of a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-22.



5. The isolated or recombinant polynucleotide of claim 4, further comprising a constitutive, inducible, or tissue-active promoter operably linked to the nucleotide sequence.
6. A cloning or expression vector comprising the isolated or recombinant polynucleotide of claim 4.
7. A cell comprising the cloning or expression vector of claim 6.
8. A transgenic plant comprising the isolated or recombinant polynucleotide of claim 4.
9. A composition produced by one or more of:
- (a) incubating one or more polynucleotide of claim 4 with a nuclease;
  - (b) incubating one or more polynucleotide of claim 4 with a restriction enzyme;
  - (c) incubating one or more polynucleotide of claim 4 with a polymerase;
  - (d) incubating one or more polynucleotide of claim 4 with a polymerase and a primer;
  - (e) incubating one or more polynucleotide of claim 4 with a cloning vector, or
  - (f) incubating one or more polynucleotide of claim 4 with a cell.
10. A composition comprising two or more different polynucleotides of claim 4.
11. An isolated or recombinant polypeptide comprising a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotide of claim 4.
12. A plant ectopically expressing an isolated polypeptide of claim 11.
13. A method for producing a plant having a modified biochemical characteristic, the method comprising altering the expression of the isolated or recombinant polynucleotide of claim 4 or the expression levels or activity of a polypeptide of claim 11 in a plant, thereby producing a modified plant, and selecting the modified plant for a modified biochemical characteristic thereby providing the modified plant with a modified biochemical characteristic.
14. The method of claim 13, wherein the polynucleotide is a polynucleotide of claim 4.

15. A method of identifying a factor that is modulated by or interacts with a polypeptide encoded by a polynucleotide of claim 4, the method comprising:

- (a) expressing a polypeptide encoded by the polynucleotide in a plant; and
- (b) identifying at least one factor that is modulated by or interacts with the polypeptide.

5

16. The method of claim 15, wherein the identifying is performed by detecting binding by the polypeptide to a promoter sequence, or detecting interactions between an additional protein and the polypeptide in a yeast two hybrid system.

10 17. The method of claim 15, wherein the identifying is performed by detecting expression of a factor by hybridization to a microarray, subtractive hybridization or differential display.

18. A method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest, the method comprising:

- 15 (a) placing the molecule in contact with a plant comprising the polynucleotide or polypeptide encoded by the polynucleotide of claim 4; and,
- (b) monitoring one or more of:
  - (i) expression level of the polynucleotide in the plant;
  - (ii) expression level of the polypeptide in the plant;
  - 20 (iii) modulation of an activity of the polypeptide in the plant; or
  - (iv) modulation of an activity of the polynucleotide in the plant.

19. An integrated system, computer or computer readable medium comprising one or more character strings corresponding to a polynucleotide of claim 4, or to a polypeptide encoded by the polynucleotide.

25

20. The integrated system, computer or computer readable medium of claim 19, further comprising a link between said one or more sequence strings to a modified plant biochemical characteristics phenotype.

30

21. A method of identifying a sequence similar or homologous to one or more polynucleotides of claim 4, or one or more polypeptides encoded by the polynucleotides, the method comprising:

- (a) providing a sequence database; and,

(b) querying the sequence database with one or more target sequences corresponding to the one or more polynucleotides or to the one or more polypeptides to identify one or more sequence members of the database that display sequence similarity or homology to one or more of the one or more target sequences.

5

22. The method of claim 21, wherein the querying comprises aligning one or more of the target sequences with one or more of the one or more sequence members in the sequence database.

10 23. The method of claim 21, wherein the querying comprises identifying one or more of the one or more sequence members of the database that meet a user-selected identity criteria with one or more of the target sequences.

24. The method of claim 21, further comprising linking the one or more of the  
15 polynucleotides of claim 4, or encoded polypeptides, to a modified plant biochemical characteristics phenotype.

25. A plant comprising altered expression levels of an isolated or recombinant polynucleotide of claim 4.

20

26. A plant comprising altered expression levels or the activity of an isolated or recombinant polypeptide of claim 11.

27. A plant lacking a nucleotide sequence encoding a polynucleotide of claim 11.

25

Figure 1

SEQ ID No.	GID	cDNA or protein	conserved domain
1	G214	cDNA	
2	G214	protein	22-71
3	G231	cDNA	
4	G231	protein	14-118
5	G274	cDNA	
6	G274	protein	108-572
7	G307	cDNA	
8	G307	protein	323-339
9	G346	cDNA	
10	G346	protein	196-221
11	G598	cDNA	
12	G598	protein	205-263
13	G605	cDNA	
14	G605	protein	132-143
15	G777	cDNA	
16	G777	protein	47-101
17	G869	cDNA	
18	G869	protein	109-177
19	G1133	cDNA	
20	G1133	protein	256-326
21	G1266	cDNA	
22	G1266	protein	79-147
23	G1324	cDNA	
24	G1324	protein	20-118
25	G1337	cDNA	
26	G1337	protein	9-75
27	G975	cDNA	
28	G975	protein	4-71

Figure 2

SEQ ID No.	GID	homolog	cDNA or protein	conserved domain
29	G680	homolog of G214	cDNA	
30	G680	homolog of G214	protein	24-70
31	G883	homolog of G274	cDNA	
32	G883	homolog of G274	protein	245-302
33	G1855	homolog of G274	cDNA	
34	G1855	homolog of G274	protein	entire protein
35	G1190	homolog of G274	cDNA	
36	G1190	homolog of G274	protein	entire protein
37	G308	homolog of G307	cDNA	
38	G308	homolog of G307	protein	270-274
39	G1944	homolog of G605	cDNA	
40	G1944	homolog of G605	protein	87-100
41	G326	homolog of G1337	cDNA	
42	G326	homolog of G1337	protein	11-94, 354-400
43	G1387	homolog of G975	cDNA	
44	G1387	homolog of G975	protein	4-71

Figure 3A

SEQ ID No.	GID	Genbank NID	P-value	Species
1	G214	8170933	8.80E-35	Lycopersicon esculentum
1	G214	9205339	1.20E-27	Glycine max
1	G214	8577344	1.80E-23	Zea mays
1	G214	9119112	2.40E-18	Medicago truncatula
1	G214	7660673	4.80E-15	Sorghum bicolor
1	G214	8213273	4.40E-14	Oryza sativa
1	G214	3325786	4.70E-10	Gossypium hirsutum
1	G214	9435251	1.50E-09	Hordeum vulgare
1	G214	9411569	6.80E-09	Triticum aestivum
1	G214	7614730	3.00E-07	Lotus japonicus
3	G231	6651291	7.80E-71	Pimpinella brachycarpa
3	G231	1430845	1.90E-62	Lycopersicon esculentum
3	G231	5268844	1.40E-61	Zea mays
3	G231	7561750	3.90E-60	Medicago truncatula
3	G231	1945282	3.30E-59	Oryza sativa
3	G231	22637	9.80E-49	Physcomitrella patens
3	G231	437326	2.00E-48	Gossypium hirsutum
3	G231	20562	3.40E-48	Petunia x hybrida
3	G231	4886263	5.00E-48	Antirrhinum majus
3	G231	8379692	1.50E-47	Gossypium arboreum
5	G274	6752887	1.70E-231	Malus domestica
5	G274	5734616	1.20E-140	Oryza sativa
5	G274	8996178	5.40E-96	Suaeda maritima subsp. salsa
5	G274	6654657	1.50E-89	Medicago truncatula
5	G274	8105703	2.30E-88	Lycopersicon esculentum
5	G274	7625402	4.00E-87	Gossypium arboreum
5	G274	7588836	2.10E-82	Glycine max
5	G274	5045979	1.30E-76	Gossypium hirsutum
5	G274	7324635	1.90E-71	Lycopersicon pennellii
5	G274	8903627	3.60E-63	Hordeum vulgare
7	G307	5640156	3.80E-151	Triticum aestivum
7	G307	5640154	1.00E-101	Zea mays
7	G307	6970471	1.70E-97	Oryza sativa
7	G307	7718432	4.00E-82	Medicago truncatula
7	G307	8330344	7.90E-78	Mesembryanthemum crystallinum
7	G307	5047560	1.00E-72	Gossypium hirsutum
7	G307	7588689	2.70E-69	Glycine max
7	G307	7623983	2.20E-64	Gossypium arboreum
7	G307	7780253	9.30E-59	Lotus japonicus
7	G307	6733213	1.90E-51	Lycopersicon esculentum
9	G346	4387642	5.90E-28	Lycopersicon esculentum
9	G346	7627902	1.50E-27	Gossypium arboreum
9	G346	8335147	6.40E-27	Oryza sativa
9	G346	8529362	9.10E-27	Medicago truncatula
9	G346	403305	2.30E-26	Nicotiana tabacum
9	G346	9299618	2.50E-26	Sorghum bicolor
9	G346	5056246	7.80E-26	Brassica rapa subsp. pekinensis
9	G346	6827291	6.80E-25	Zea mays
9	G346	6567406	1.90E-24	Glycine max
9	G346	9425896	1.20E-21	Triticum turgidum subsp. durum
11	G598	8102670	1.30E-43	Zea mays
11	G598	4382198	9.80E-42	Lycopersicon esculentum

Figure 3B

SEQ ID No.	GID	Genbank NID	P-value	Species
11	G598	7553316	8.00E-38	Sorghum bicolor
11	G598	9445834	3.10E-36	Triticum aestivum
11	G598	7332502	8.80E-30	Oryza sativa
11	G598	9056816	1.70E-17	Medicago truncatula
11	G598	6644720	5.20E-15	Mesembryanthemum crystallinum
11	G598	3853398	2.20E-14	Populus tremula x Populus tremuloides
11	G598	9419408	6.80E-09	Hordeum vulgare
11	G598	6848223	1.40E-06	Glycine max
13	G605	7624850	4.40E-49	Gossypium arboreum
13	G605	9204125	6.50E-46	Glycine max
13	G605	2213533	5.50E-33	Pisum sativum
13	G605	7009437	1.40E-28	Zea mays
13	G605	8104258	3.50E-28	Lycopersicon esculentum
13	G605	7536402	4.10E-28	Sorghum bicolor
13	G605	3107210	1.60E-22	Oryza sativa
13	G605	7784135	9.20E-20	Lotus japonicus
13	G605	4165182	8.30E-18	Antirrhinum majus
13	G605	6555294	8.10E-17	Pinus taeda
15	G777	8172576	3.10E-29	Medicago truncatula
15	G777	8331320	4.60E-17	Mesembryanthemum crystallinum
15	G777	8106138	3.00E-16	Lycopersicon esculentum
15	G777	5046832	1.20E-14	Gossypium hirsutum
15	G777	6918785	1.70E-13	Zea mays
15	G777	5666914	1.30E-07	Glycine max
15	G777	8856987	0.98	Oryza sativa
15	G777	8404755	1	Hordeum vulgare
17	G869	2213784	1.30E-19	Lycopersicon esculentum
17	G869	3065894	7.30E-19	Nicotiana tabacum
17	G869	8570080	4.20E-18	Oryza sativa
17	G869	7560260	1.50E-17	Medicago truncatula
17	G869	7534890	5.20E-14	Sorghum bicolor
17	G869	6455322	1.10E-13	Glycine max
17	G869	9362061	2.70E-13	Triticum aestivum
17	G869	7788764	5.70E-13	Lotus japonicus
17	G869	7624302	2.50E-12	Gossypium arboreum
17	G869	3858036	2.80E-12	Populus balsamifera subsp. trichocarpa
19	G1133	8070726	1.30E-16	Solanum tuberosum
19	G1133	6848196	1.60E-16	Glycine max
19	G1133	7570922	3.60E-13	Medicago truncatula
19	G1133	9434859	1.90E-12	Lycopersicon esculentum
19	G1133	5704484	0.005	Oryza sativa
19	G1133	902661	0.0081	Hordeum vulgare
19	G1133	8666194	0.0086	Pinus taeda
19	G1133	5725018	0.14	Brassica rapa subsp. pekinensis
19	G1133	7501051	0.64	Gossypium arboreum
19	G1133	7747388	0.98	Lotus japonicus
21	G1266	1732405	1.50E-50	Nicotiana tabacum
21	G1266	7145976	2.50E-38	Glycine max
21	G1266	3326366	1.00E-37	Gossypium hirsutum
21	G1266	5762854	6.90E-37	Lotus japonicus
21	G1266	7560749	9.10E-34	Medicago truncatula
21	G1266	7934594	6.60E-33	Euphorbia esula
21	G1266	9431305	2.10E-28	Lycopersicon esculentum

Figure 3C

SEQ ID No.	GID	Genbank NID	P-value	Species
21	G1266	7528275	5.40E-21	Mesembryanthemum crystallinum
21	G1266	6478844	4.10E-20	Matricaria chamomilla
21	G1266	7627061	4.20E-20	Gossypium arboreum
23	G1324	2921337	2.30E-54	Gossypium hirsutum
23	G1324	5891412	3.50E-52	Lycopersicon esculentum
23	G1324	8528843	7.20E-50	Medicago truncatula
23	G1324	1002797	5.40E-49	Craterostigma plantagineum
23	G1324	5666961	3.90E-44	Glycine max
23	G1324	7244640	1.70E-42	Mentha x piperita
23	G1324	1841474	3.00E-42	Pisum sativum
23	G1324	4979554	1.30E-39	Oryza sativa
23	G1324	9363368	3.00E-32	Triticum aestivum
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25	G1337	3341722	1.60E-17	Raphanus sativus
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27	G975	19506	4.70E-14	Lupinus polyphyllus
27	G975	6799584	5.30E-14	Medicago truncatula
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31	G883	6799932	1.40E-31	Medicago truncatula
31	G883	5456433	4.30E-31	Zea mays
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31	G883	1432055	2.00E-27	Petroselinum crispum



Figure 3D

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33	G1855	8996178	1.80E-78	Suaeda maritima subsp. salsa
33	G1855	7625402	1.60E-77	Gossypium arboreum
33	G1855	8903627	3.80E-74	Hordeum vulgare
33	G1855	6654657	2.20E-70	Medicago truncatula
33	G1855	8090141	4.50E-64	Sorghum bicolor
33	G1855	9028645	6.30E-64	Zea mays
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35	G1190	4380101	5.50E-88	Lycopersicon esculentum
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35	G1190	8666639	5.50E-75	Pinus taeda
35	G1190	8088688	3.40E-72	Sorghum bicolor
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37	G308	7588689	1.90E-68	Glycine max
37	G308	7623983	2.90E-62	Gossypium arboreum
37	G308	7780253	1.10E-57	Lotus japonicus
37	G308	6733213	3.70E-48	Lycopersicon esculentum
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39	G1944	4165182	7.10E-17	Antirrhinum majus
39	G1944	6555294	2.90E-16	Pinus taeda
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41	G326	7571599	4.90E-30	Medicago truncatula
41	G326	7232283	6.30E-28	Glycine max
41	G326	7323708	6.00E-27	Lycopersicon hirsutum
41	G326	4091805	2.30E-19	Malus domestica
41	G326	6917805	6.50E-19	Lycopersicon pennellii
41	G326	3341722	2.50E-18	Raphanus sativus
41	G326	4557092	7.50E-18	Pinus radiata
41	G326	2303680	4.70E-17	Brassica napus
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43	G1387	8103850	5.20E-46	Lycopersicon esculentum
43	G1387	5056299	1.10E-20	Brassica rapa subsp. pekinensis

Figure 3E

SEQ ID No.	GID	Genbank NID	P-value	Species
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43	G1387	7766740	4.70E-14	Medicago truncatula
43	G1387	9427282	1.40E-12	Triticum aestivum
43	G1387	3857766	3.40E-12	Populus balsamifera subsp. trichocarpa
43	G1387	19506	4.60E-12	Lupinus polyphyllus
43	G1387	7273843	2.20E-11	Oryza sativa

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caa gag caa gac cga gct atg aag ttc ccg aga gag aac atg att tac 513  
Gln Glu Gln Asp Arg Ala Met Lys Phe Pro Arg Glu Asn Met Ile Tyr  
100 105 110  
aga gag aga cat tgt cct cct gat aat gag aag ctg cgt tgt ctt gtt 561  
Arg Glu Arg His Cys Pro Pro Asp Asn Glu Lys Leu Arg Cys Leu Val  
115 120 125 130  
cca gct cct aaa ggg tat atg act cct ttc cct tgg cct aaa agc aga 609  
Pro Ala Pro Lys Gly Tyr Met Thr Pro Phe Pro Trp Pro Lys Ser Arg  
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gat tat gtt cac tat gct aat gct cct ttc aag agc ttg act gtc gaa 657  
Asp Tyr Val His Tyr Ala Asn Ala Pro Phe Lys Ser Leu Thr Val Glu  
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aaa gct gga cag aat tgg gtt cag ttt caa ggg aat gtg ttt aaa ttc 705  
Lys Ala Gly Gln Asn Trp Val Gln Phe Gln Gly Asn Val Phe Lys Phe  
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cct ggt gga gga act atg ttt cct caa ggt gct gat gcg tat att gaa 753  
Pro Gly Gly Gly Thr Met Phe Pro Gln Gly Ala Asp Ala Tyr Ile Glu  
180 185 190  
gag cta gct tct gtt atc cct atc aaa gat ggc tct gtt aga acc gca 801  
Glu Leu Ala Ser Val Ile Pro Ile Lys Asp Gly Ser Val Arg Thr Ala  
195 200 205 210

## MBI-20 Sequence Listing.ST25

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caa gtc cag ttt gcg ctt gag aga ggt gtt cca gcg att atc gct gtt Gln Val Gln Phe Ala Leu Glu Arg Gly Val Pro Ala Ile Ile Ala Val 245 250 255	945
ctt gga tca atc ctt ctt cct tac cct gca aga gcc ttt gac atg gct Leu Gly Ser Ile Leu Leu Pro Tyr Pro Ala Arg Ala Phe Asp Met Ala 260 265 270	993
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tcg ggt cct cca atc aac tgg aag aca tgg cac aag acg tgg aac cga Ser Gly Pro Pro Ile Asn Trp Lys Thr Trp His Lys Thr Trp Asn Arg 310 315 320	1137
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cca agt atc tct aaa ggt ttg att aat ggc gtc gac gag gaa tca tac Pro Ser Ile Ser Lys Gly Leu Ile Asn Gly Val Asp Glu Glu Ser Tyr 420 425 430	1473
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aac gcc ggt ctt ggt gga ttc gct gct gcg ctt gaa tcg cct aaa tcg Asn Ala Gly Leu Gly Gly Phe Ala Ala Ala Leu Glu Ser Pro Lys Ser 470 475 480	1617
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MBI-20 Sequence Listing.ST25

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ttc agc ttg tat cag cac agc tgc aaa ctt gag gat att ctt ctt gaa			1809
Phe Ser Leu Tyr Gln His Ser Cys Lys Leu Glu Asp Ile Leu Leu Glu			
	535	540	545
act gat cgg att tta cga ccg gaa ggg att gtg att ttc cgg gat gag			1857
Thr Asp Arg Ile Leu Arg Pro Glu Gly Ile Val Ile Phe Arg Asp Glu			
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gtt gat gtt ttg aat gat gtg agg aag atc gtt gat gga atg aga tgg			1905
Val Asp Val Leu Asn Asp Val Arg Lys Ile Val Asp Gly Met Arg Trp			
	565	570	575
gat act aag tta atg gat cat gaa gac ggt cct ctc gtg ccg gag aag			1953
Asp Thr Lys Leu Met Asp His Glu Asp Gly Pro Leu Val Pro Glu Lys			
	580	585	590
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Ile Leu Val Ala Thr Lys Gln Tyr Trp Val Ala Gly Asp Asp Gly Asn			
595	600	605	610
aat tct ccg tcg tct tct aat agt gaa gaa gaa taa aacaaaaaca			2047
Asn Ser Pro Ser Ser Ser Asn Ser Glu Glu			
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cttatcaaaa aaggaaggaa tcagaatttc cattaaagaa aggtgtcaaa aaaaagttgt			2167
aaaactatat agtagtgatc aagacgaata tgtgcattta tgttttattt ttgttcccta			2227
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Gly Ala Trp Gln Lys Ser Gly Phe Gly Lys Gly Asp Ser Ile Ala Met			
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Glu Ile Thr Lys Gln Ala Gln Cys Thr Asp Ile Val Thr Asp Leu Asp			
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Phe Glu Pro His His Asn Thr Val Lys Ile Pro His Lys Ala Asp Pro			
65	70	75	80
Lys Pro Val Ser Phe Lys Pro Cys Asp Val Lys Leu Lys Asp Tyr Thr			
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Pro Cys Gln Glu Gln Asp Arg Ala Met Lys Phe Pro Arg Glu Asn Met			

## MBI-20 Sequence Listing.ST25

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Ser Arg Asp Tyr Val His Tyr Ala Asn Ala Pro Phe Lys Ser Leu Thr
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Val Glu Lys Ala Gly Gln Asn Trp Val Gln Phe Gln Gly Asn Val Phe
165 170 175
Lys Phe Pro Gly Gly Gly Thr Met Phe Pro Gln Gly Ala Asp Ala Tyr
180 185 190
Ile Glu Glu Leu Ala Ser Val Ile Pro Ile Lys Asp Gly Ser Val Arg
195 200 205
Thr Ala Leu Asp Thr Gly Cys Gly Val Ala Ser Trp Gly Ala Tyr Met
210 215 220
Leu Lys Arg Asn Val Leu Thr Met Ser Phe Ala Pro Arg Asp Asn His
225 230 235 240
Glu Ala Gln Val Gln Phe Ala Leu Glu Arg Gly Val Pro Ala Ile Ile
245 250 255
Ala Val Leu Gly Ser Ile Leu Leu Pro Tyr Pro Ala Arg Ala Phe Asp
260 265 270
Met Ala Gln Cys Ser Arg Cys Leu Ile Pro Trp Thr Ala Asn Glu Gly
275 280 285
Thr Tyr Leu Met Glu Val Asp Arg Val Leu Arg Pro Gly Gly Tyr Trp
290 295 300
Val Leu Ser Gly Pro Pro Ile Asn Trp Lys Thr Trp His Lys Thr Trp
305 310 315 320
Asn Arg Thr Lys Ala Glu Leu Asn Ala Glu Gln Lys Arg Ile Glu Gly
325 330 335
Ile Ala Glu Ser Leu Cys Trp Glu Lys Lys Tyr Glu Lys Gly Asp Ile
340 345 350
Ala Ile Phe Arg Lys Lys Ile Asn Asp Arg Ser Cys Asp Arg Ser Thr
355 360 365
Pro Val Asp Thr Cys Lys Arg Lys Asp Thr Asp Asp Val Trp Tyr Lys
370 375 380
Glu Ile Glu Thr Cys Val Thr Pro Phe Pro Lys Val Ser Asn Glu Glu
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## MBI-20 Sequence Listing.ST25

Glu Val Ala Gly Gly Lys Leu Lys Lys Phe Pro Glu Arg Leu Phe Ala  
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 Val Pro Pro Ser Ile Ser Lys Gly Leu Ile Asn Gly Val Asp Glu Glu  
 420 425 430  
 Ser Tyr Gln Glu Asp Ile Asn Leu Trp Lys Lys Arg Val Thr Gly Tyr  
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 Lys Arg Ile Asn Arg Leu Ile Gly Ser Thr Arg Tyr Arg Asn Val Met  
 450 455 460  
 Asp Met Asn Ala Gly Leu Gly Gly Phe Ala Ala Ala Leu Glu Ser Pro  
 465 470 475 480  
 Lys Ser Trp Val Met Asn Val Ile Pro Thr Ile Asn Lys Asn Thr Leu  
 485 490 495  
 Ser Val Val Tyr Glu Arg Gly Leu Ile Gly Ile Tyr His Asp Trp Cys  
 500 505 510  
 Glu Gly Phe Ser Thr Tyr Pro Arg Thr Tyr Asp Phe Ile His Ala Ser  
 515 520 525  
 Gly Val Phe Ser Leu Tyr Gln His Ser Cys Lys Leu Glu Asp Ile Leu  
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 Asp Glu Val Asp Val Leu Asn Asp Val Arg Lys Ile Val Asp Gly Met  
 565 570 575  
 Arg Trp Asp Thr Lys Leu Met Asp His Glu Asp Gly Pro Leu Val Pro  
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 1 5 10 15  
 act: tct tct tct tca tca tca atc tct aaa gat aag atg atg atg gtg 96  
 Thr: Ser Ser Ser Ser Ser Ile Ser Lys Asp Lys Met Met Met Val  
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## MBI-20 Sequence Listing.ST25

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ggt tta ggt tac aaa gtt agg tca tcg gag atg gcg gag gtt gct ttg Val Leu Gly Tyr Lys Val Arg Ser Ser Glu Met Ala Glu Val Ala Leu 50 55 60	192
aaa ctc gaa caa tta gag acg atg atg agt aat gtt caa gaa gat ggt Lys Leu Glu Gln Leu Glu Thr Met Met Ser Asn Val Gln Glu Asp Gly 65 70 75 80	240
tta tct cat ctc gcg acg gat act gtt cat tat aat ccg tcg gag ctt Leu Ser His Leu Ala Thr Asp Thr Val His Tyr Asn Pro Ser Glu Leu 85 90 95	288
tat tct tgg ctt gat aat atg ctc tct gag ctt aat cct cct cct ctt Tyr Ser Trp Leu Asp Asn Met Leu Ser Glu Leu Asn Pro Pro Pro Leu 100 105 110	336
ccg gcg agt tct aac ggt tta gat ccg gtt ctt cct tcg ccg gag att Pro Ala Ser Ser Asn Gly Leu Asp Pro Val Leu Pro Ser Pro Glu Ile 115 120 125	384
tgt ggt ttt ccg gct tcg gat tat gac ctt aaa gtc att ccc gga aac Cys Gly Phe Pro Ala Ser Asp Tyr Asp Leu Lys Val Ile Pro Gly Asn 130 135 140	432
gcg att tat cag ttt ccg gcg att gat tct tcg tct tcg tcg aat aat Ala Ile Tyr Gln Phe Pro Ala Ile Asp Ser Ser Ser Ser Ser Asn Asn 145 150 155 160	480
cag aac aag cgt ttg aaa tca tgc tcg agt cct gat tct atg gtt aca Gln Asn Lys Arg Leu Lys Ser Cys Ser Ser Pro Asp Ser Met Val Thr 165 170 175	528
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ctt atg gct tgt gca gaa gca atc cag cag aac aat ttg act cta gcg Leu Met Ala Cys Ala Glu Ala Ile Gln Gln Asn Asn Leu Thr Leu Ala 225 230 235 240	720
gaa gct ctt gtg aag caa atc gga tgc tta gct gtg tct caa gcc gga Glu Ala Leu Val Lys Gln Ile Gly Cys Leu Ala Val Ser Gln Ala Gly 245 250 255	768
gct atg aga aaa gtg gct act tac ttc gcc gaa gct tta gct cgg cgg Ala Met Arg Lys Val Ala Thr Tyr Phe Ala Glu Ala Leu Ala Arg Arg 260 265 270	816
atc tac cgt ctc tct ccg ccg cag aat cag atc gat cat tgt ctc tcc Ile Tyr Arg Leu Ser Pro Pro Gln Asn Gln Ile Asp His Cys Leu Ser 275 280 285	864
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gct cac ttc acg gcg aac caa gcg att ctc gaa gct ttt gaa ggt aag Ala His Phe Thr Ala Asn Gln Ala Ile Leu Glu Ala Phe Glu Gly Lys 305 310 315 320	960
aag aga gta cac gtc att gat ttc tcg atg aac caa ggt ctt caa tgg Lys Arg Val His Val Ile Asp Phe Ser Met Asn Gln Gly Leu Gln Trp	1008

## MBI-20 Sequence Listing.ST25

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ttc cgg tta acc gga att ggt cca ccg gcg ccg gat aat tct gat cat Phe Arg Leu Thr Gly Ile Gly Pro Pro Ala Pro Asp Asn Ser Asp His 355 360 365			1104
ctt cat gaa gtt ggt tgt aaa tta gct cag ctt gcg gag gcg att cac Leu His Glu Val Gly Cys Lys Leu Ala Gln Leu Ala Glu Ala Ile His 370 375 380			1152
gta gaa ttc gaa tac cgt gga ttc gtt gct aac agc tta gcc gat ctc Val Glu Phe Glu Tyr Arg Gly Phe Val Ala Asn Ser Leu Ala Asp Leu 385 390 395 400			1200
gat gct tcg atg ctt gag ctt aga ccg agc gat acg gaa gct gtt gcg Asp Ala Ser Met Leu Glu Leu Arg Pro Ser Asp Thr Glu Ala Val Ala 405 410 415			1248
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ata gag aaa gtt ctc ggc gtt gtg aaa cag att aaa ccg gtg att ttc Ile Glu Lys Val Leu Gly Val Val Lys Gln Ile Lys Pro Val Ile Phe 435 440 445			1344
acg gtg gtt gag caa gaa tcg aac cat aac gga ccg gtt ttc tta gac Thr Val Val Glu Gln Glu Ser Asn His Asn Gly Pro Val Phe Leu Asp 450 455 460			1392
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ggc tta gcg ccg gca cat ctt ggg tct aac gcg ttt aag caa gcg agt Gly Leu Ala Pro Ala His Leu Gly Ser Asn Ala Phe Lys Gln Ala Ser 530 535 540			1632
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Lys	Lys	Glu	Glu	Asp	Gly	Gly	Gly	Asn	Met	Asp	Asp	Glu	Leu	Leu	Ala	
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Val	Leu	Gly	Tyr	Lys	Val	Arg	Ser	Ser	Glu	Met	Ala	Glu	Val	Ala	Leu	
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Lys	Leu	Glu	Gln	Leu	Glu	Thr	Met	Met	Ser	Asn	Val	Gln	Glu	Asp	Gly	
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Leu	Ser	His	Leu	Ala	Thr	Asp	Thr	Val	His	Tyr	Asn	Pro	Ser	Glu	Leu	
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Pro	Ala	Ser	Ser	Asn	Gly	Leu	Asp	Pro	Val	Leu	Pro	Ser	Pro	Glu	Ile	
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Cys	Gly	Phe	Pro	Ala	Ser	Asp	Tyr	Asp	Leu	Lys	Val	Ile	Pro	Gly	Asn	
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Gln	Asn	Lys	Arg	Leu	Lys	Ser	Cys	Ser	Ser	Pro	Asp	Ser	Met	Val	Thr	
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			180					185					190			
Thr	Thr	Thr	Thr	Thr	Thr	Thr	Thr	Ala	Ala	Ala	Glu	Ser	Thr	Arg	Ser	
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Val	Ile	Leu	Val	Asp	Ser	Gln	Glu	Asn	Gly	Val	Arg	Leu	Val	His	Ala	
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Ile	Tyr	Arg	Leu	Ser	Pro	Pro	Gln	Asn	Gln	Ile	Asp	His	Cys	Leu	Ser	
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Asp	Thr	Leu	Gln	Met	His	Phe	Tyr	Glu	Thr	Cys	Pro	Tyr	Leu	Lys	Phe	
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## MBI-20 Sequence Listing.ST25

Ala His Phe Thr Ala Asn Gln Ala Ile Leu Glu Ala Phe Glu Gly Lys  
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Pro Ala Leu Met Gln Ala Leu Ala Leu Arg Glu Gly Gly Pro Pro Thr  
 340 345 350

Phe Arg Leu Thr Gly Ile Gly Pro Pro Ala Pro Asp Asn Ser Asp His  
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Leu His Glu Val Gly Cys Lys Leu Ala Gln Leu Ala Glu Ala Ile His  
 370 375 380

Val Glu Phe Glu Tyr Arg Gly Phe Val Ala Asn Ser Leu Ala Asp Leu  
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Asp Ala Ser Met Leu Glu Leu Arg Pro Ser Asp Thr Glu Ala Val Ala  
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Val Asn Ser Val Phe Glu Leu His Lys Leu Leu Gly Arg Pro Gly Gly  
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Ile Glu Lys Val Leu Gly Val Val Lys Gln Ile Lys Pro Val Ile Phe  
 435 440 445

Thr Val Val Glu Gln Glu Ser Asn His Asn Gly Pro Val Phe Leu Asp  
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Arg Phe Thr Glu Ser Leu His Tyr Tyr Ser Thr Leu Phe Asp Ser Leu  
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Glu Gly Val Pro Asn Ser Gln Asp Lys Val Met Ser Glu Val Tyr Leu  
 485 490 495

Gly Lys Gln Ile Cys Asn Leu Val Ala Cys Glu Gly Pro Asp Arg Val  
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Glu Arg His Glu Thr Leu Ser Gln Trp Gly Asn Arg Phe Gly Ser Ser  
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Gly Leu Ala Pro Ala His Leu Gly Ser Asn Ala Phe Lys Gln Ala Ser  
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Met Leu Leu Ser Val Phe Asn Ser Gly Gln Gly Tyr Arg Val Glu Glu  
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## MBI-20 Sequence Listing.ST25

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&lt;223&gt; G346

&lt;400&gt; 9

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act cgc cgg aaa act gga tta cgg cca aca gac tcc ttc ggt ctc ttt	144
Thr Arg Arg Lys Thr Gly Leu Arg Pro Thr Asp Ser Phe Gly Leu Phe	
35 40 45	
aat acc gac gac ctt gga gtg gtt gaa gaa gag gat ttg gaa tgg att	192
Asn Thr Asp Asp Leu Gly Val Val Glu Glu Glu Asp Leu Glu Trp Ile	
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65 70 75 80	
ccg tcg gag cat ttt cct ata acg tct ctt ctg gaa aga gaa gcg act	288
Pro Ser Glu His Phe Pro Ile Thr Ser Leu Leu Glu Arg Glu Ala Thr	
85 90 95	
gag gta aaa cag ctg agt ccg gtt tca gta ctt gag acg agt agc cat	336
Glu Val Lys Gln Leu Ser Pro Val Ser Val Leu Glu Thr Ser Ser His	
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agc tcc aca acg act acc tca aac agt agc ggc gga agt aac gga agc	384
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Thr Ala Val Ala Thr Thr Thr Thr Thr Pro Thr Ile Met Ser Cys Cys	
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Gly Arg Lys Cys Gln His Cys Gly Ala Glu Lys Thr Pro Gln Trp Arg	
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gca gga cca gcg ggg cct aag act ctg tgt aac gct tgt ggc gtg agg	672
Ala Gly Pro Ala Gly Pro Lys Thr Leu Cys Asn Ala Cys Gly Val Arg	
210 215 220	
tat aag tcc ggg agg cta gtt ccg gag tat cgt cca gcg aac agt cca	720
Tyr Lys Ser Gly Arg Leu Val Pro Glu Tyr Arg Pro Ala Asn Ser Pro	
225 230 235 240	
act ttc acg gcg gag tta cat tcg aat tct cac ccg aag att gta gag	768
Thr Phe Thr Ala Glu Leu His Ser Asn Ser His Arg Lys Ile Val Glu	
245 250 255	
atg agg aag cag tat cag tcc ggt gac ggt gac ggt gat ccg aaa gat	816
Met Arg Lys Gln Tyr Gln Ser Gly Asp Gly Asp Gly Asp Arg Lys Asp	

## MBI-20 Sequence Listing.ST25

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265

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Cys Gly

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35 40 45

Asn Thr Asp Asp Leu Gly Val Val Glu Glu Glu Asp Leu Glu Trp Ile  
50 55 60

Ser Asn Lys Asn Ala Phe Pro Val Ile Glu Thr Phe Val Gly Val Leu  
65 70 75 80

Pro Ser Glu His Phe Pro Ile Thr Ser Leu Leu Glu Arg Glu Ala Thr  
85 90 95

Glu Val Lys Gln Leu Ser Pro Val Ser Val Leu Glu Thr Ser Ser His  
100 105 110

Ser Ser Thr Thr Thr Thr Ser Asn Ser Ser Gly Gly Ser Asn Gly Ser  
115 120 125

Thr Ala Val Ala Thr Thr Thr Thr Thr Pro Thr Ile Met Ser Cys Cys  
130 135 140

Val Gly Phe Lys Ala Pro Ala Lys Ala Arg Ser Lys Arg Arg Arg Thr  
145 150 155 160

Gly Arg Arg Asp Leu Arg Val Leu Trp Thr Gly Asn Glu Gln Gly Gly  
165 170 175

Ile Gln Lys Lys Lys Thr Met Thr Val Ala Ala Ala Ala Leu Ile Met  
180 185 190

Gly Arg Lys Cys Gln His Cys Gly Ala Glu Lys Thr Pro Gln Trp Arg  
195 200 205

Ala Gly Pro Ala Gly Pro Lys Thr Leu Cys Asn Ala Cys Gly Val Arg  
210 215 220

Tyr Lys Ser Gly Arg Leu Val Pro Glu Tyr Arg Pro Ala Asn Ser Pro  
225 230 235 240

Thr Phe Thr Ala Glu Leu His Ser Asn Ser His Arg Lys Ile Val Glu

## MBI-20 Sequence Listing.ST25

245

250

255

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 caattagctt cgcgatatat cagaagagat caaactactt tgatcagacc atgatcttct 180  
 tcttcttctt cttcttcttc ttcttctttt tagacgatca caattcctaa accctatttc 240  
 tcagatt atg ctg act ctt tac cat caa gaa agg tca ccg gac gcc aca 289  
 Met Leu Thr Leu Tyr His Gln Glu Arg Ser Pro Asp Ala Thr  
 1 5 10  
 agt aat gat cgc gat gag acg cca gag act gtg gtt aga gaa gtc cac 337  
 Ser Asn Asp Arg Asp Glu Thr Pro Glu Thr Val Val Arg Glu Val His  
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 gcg cta act cca gcg ccg gag gat aat tcc ccg acg atg acg gcg acg 385  
 Ala Leu Thr Pro Ala Pro Glu Asp Asn Ser Arg Thr Met Thr Ala Thr  
 35 40 45  
 cta cct cca ccg cct gct ttc cga ggc tat ttt tct cct cca agg tca 433  
 Leu Pro Pro Pro Ala Phe Arg Gly Tyr Phe Ser Pro Pro Arg Ser  
 50 55 60  
 gcg acg acg atg agc gaa gga gag aac ttc aca act ata agc aga gag 481  
 Ala Thr Thr Met Ser Glu Gly Glu Asn Phe Thr Thr Ile Ser Arg Glu  
 65 70 75  
 ttc aac gct cta gtc atc gcc gga tcc tcc atg gag aac aac gaa cta 529  
 Phe Asn Ala Leu Val Ile Ala Gly Ser Ser Met Glu Asn Asn Glu Leu  
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 atg act cgt gac gtc acg cag cgt gaa gat gag aga caa gac gag ttg 577  
 Met Thr Arg Asp Val Thr Gln Arg Glu Asp Glu Arg Gln Asp Glu Leu  
 95 100 105 110  
 atg aga atc cac gag gac acg gat cat gaa gag gaa acg aat cct tta 625  
 Met Arg Ile His Glu Asp Thr Asp His Glu Glu Glu Thr Asn Pro Leu  
 115 120 125  
 gca atc gtg ccg gat cag tat cct ggt tcg ggt ttg gat cct gga agt 673  
 Ala Ile Val Pro Asp Gln Tyr Pro Gly Ser Gly Leu Asp Pro Gly Ser  
 130 135 140  
 gat aat ggg ccg ggt cag agt cgg gtt ggg tcg acg gtg caa aga gtt 721  
 Asp Asn Gly Pro Gly Gln Ser Arg Val Gly Ser Thr Val Gln Arg Val  
 145 150 155  
 aag agg gaa gag gtg gaa gcg aag ata acg gcg tgg cag acg gca aaa 769  
 Lys Arg Glu Glu Val Glu Ala Lys Ile Thr Ala Trp Gln Thr Ala Lys  
 160 165 170

## MBI-20 Sequence Listing.ST25

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 175 180 185 190

gg: tgg ttt aat gaa caa gtt aac aag gcc aac tct tgg atg aag aaa 865  
 Gly Trp Phe Asn Glu Gln Val Asn Lys Ala Asn Ser Trp Met Lys Lys  
 195 200 205

at: gag tat aat gta ggt tca ttc aac aat cgt cta aat gag gaa gct 913  
 Ile Glu Tyr Asn Val Gly Ser Phe Asn Asn Arg Leu Asn Glu Glu Ala  
 210 215 220

aga gga gag aaa agc aaa agc gat gga gaa aac gca aaa caa tgt ggc 961  
 Arg Gly Glu Lys Ser Lys Ser Asp Gly Glu Asn Ala Lys Gln Cys Gly  
 225 230 235

gaa agc gca gag gaa agc gga gga gag aag agc gac ggc aga ggc aaa 1009  
 Glu Ser Ala Glu Glu Ser Gly Gly Glu Lys Ser Asp Gly Arg Gly Lys  
 240 245 250

gag agg gac aga ggt tgc aaa agt agt tga agttgctaatt ctcattgagag 1059  
 Glu Arg Asp Arg Gly Cys Lys Ser Ser  
 255 260

ccttggacg tctcctgcc aaacgctcct tcttctcttt ctctaattt ttagttatat 1119  
 caaaccatta aattaaacag tactcggttat atatctagtt agtaaacaaa ggggcagttt 1179  
 tatagctcat gtacacataa ttgagagtgt agtactgttg tgtcaaa 1226

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<400> 12

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Thr Pro Ala Pro Glu Asp Asn Ser Arg Thr Met Thr Ala Thr Leu Pro  
 35 40 45

Pro Pro Pro Ala Phe Arg Gly Tyr Phe Ser Pro Pro Arg Ser Ala Thr  
 50 55 60

Thr Met Ser Glu Gly Glu Asn Phe Thr Thr Ile Ser Arg Glu Phe Asn  
 65 70 75 80

Ala Leu Val Ile Ala Gly Ser Ser Met Glu Asn Asn Glu Leu Met Thr  
 85 90 95

Arg Asp Val Thr Gln Arg Glu Asp Glu Arg Gln Asp Glu Leu Met Arg  
 100 105 110

Ile His Glu Asp Thr Asp His Glu Glu Glu Thr Asn Pro Leu Ala Ile  
 115 120 125

Val Pro Asp Gln Tyr Pro Gly Ser Gly Leu Asp Pro Gly Ser Asp Asn  
 130 135 140

Gly Pro Gly Gln Ser Arg Val Gly Ser Thr Val Gln Arg Val Lys Arg

145	150	155	160
Glu Glu Val Glu Ala Lys Ile Thr Ala Trp Gln Thr Ala Lys Leu Ala	165	170	175
Lys Ile Asn Asn Arg Phe Lys Arg Glu Asp Ala Val Ile Asn Gly Trp	180	185	190
Phe Asn Glu Gln Val Asn Lys Ala Asn Ser Trp Met Lys Lys Ile Glu	195	200	205
Tyr Asn Val Gly Ser Phe Asn Asn Arg Leu Asn Glu Glu Ala Arg Gly	210	215	220
Glu Lys Ser Lys Ser Asp Gly Glu Asn Ala Lys Gln Cys Gly Glu Ser	225	230	235
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Met Glu Thr Thr Gly Glu Val Val Lys Thr Thr Thr Gly			
1 5 10			
agc gac gga ggc gtt acg gtg gtg aga tcc aac gcg ccg tca gac ttc			158
Ser Asp Gly Gly Val Thr Val Val Arg Ser Asn Ala Pro Ser Asp Phe	15	20	25
cac atg gct ccg agg tca gaa act tca aac aca cct ccc aac tcc gtc			206
His Met Ala Pro Arg Ser Glu Thr Ser Asn Thr Pro Pro Asn Ser Val	30	35	40
45			
gct cct cct cct cct cca ccg ccg caa aac tcc ttt act ccg tcg gcg			254
Ala Pro Pro Pro Pro Pro Pro Gln Asn Ser Phe Thr Pro Ala	50	55	60
gct atg gat ggt ttc tca agc gga ccg ata aag aag aga cgt ggg cgc			302
Ala Met Asp Gly Phe Ser Ser Gly Pro Ile Lys Lys Arg Arg Gly Arg	65	70	75
cct agg aag tac gga cac gac gga gca gcg gtg acg cta tct ccg aat			350
Pro Arg Lys Tyr Gly His Asp Gly Ala Ala Val Thr Leu Ser Pro Asn	80	85	90
ccg ata tca tca gcc gca cca acg act tct cac gtc atc gat ttc tcg			398
Pro Ile Ser Ser Ala Ala Pro Thr Thr Ser His Val Ile Asp Phe Ser	95	100	105
acg aca tcg gag aaa cgt ggc aaa atg aaa cca gca act cca act cca			446
Thr Thr Ser Glu Lys Arg Gly Lys Met Lys Pro Ala Thr Pro Thr Pro			

MBI-20 Sequence Listing.ST25

110	115	120	125	
agc tca ttc atc agg cca aag tac cag gtc gag aat tta ggt gaa tgg				494
Ser Ser Phe Ile Arg Pro Lys Tyr Gln Val Glu Asn Leu Gly Glu Trp	130	135	140	
tcg cct tcc tct gcc gcc gct aat ttc acg ccg cat att att acg gtg				542
Ser Pro Ser Ser Ala Ala Ala Asn Phe Thr Pro His Ile Ile Thr Val	145	150	155	
aat gca gcc gag gac gtt acg aag agg ata ata tca ttt tct caa caa				590
Asn Ala Gly Glu Asp Val Thr Lys Arg Ile Ile Ser Phe Ser Gln Gln	160	165	170	
ggg tct cta gct att tgc gtt tta tgc gca aac ggt gtc gtt tcg agc				638
Gly Ser Leu Ala Ile Cys Val Leu Cys Ala Asn Gly Val Val Ser Ser	175	180	185	
gtt aca ctt cgt cag cct gat tca tct ggt ggt aca ttg acc tat gag				686
Val Thr Leu Arg Gln Pro Asp Ser Ser Gly Gly Thr Leu Thr Tyr Glu	190	195	200	205
ggg cgg ttt gag ata ttg tca cta tct gga aca ttc atg cct agt gac				734
Gly Arg Phe Glu Ile Leu Ser Leu Ser Gly Thr Phe Met Pro Ser Asp	210	215	220	
tca gac ggg aca cga agc aga aca gcc ggg atg agc gtg tcg ctt gct				782
Ser Asp Gly Thr Arg Ser Arg Thr Gly Gly Met Ser Val Ser Leu Ala	225	230	235	
agc cct gat gga cgt gta gta ggt ggt ggt gtt gct gcc ttg ctg gtt				830
Ser Pro Asp Gly Arg Val Val Gly Gly Gly Val Ala Gly Leu Leu Val	240	245	250	
gca gcc act cct att caa gtg gtt gta gga act ttc tta ggt gga aca				878
Ala Ala Thr Pro Ile Gln Val Val Val Gly Thr Phe Leu Gly Gly Thr	255	260	265	
aac cag caa gaa cag aca ccg aag ccg cat aac cac aac ttc atg tct				926
Asn Gln Gln Glu Gln Thr Pro Lys Pro His Asn His Asn Phe Met Ser	270	275	280	285
tct cca tta atg cca act tct tcg aat gta gct gat cat cga acc atc				974
Ser Pro Leu Met Pro Thr Ser Ser Asn Val Ala Asp His Arg Thr Ile	290	295	300	
cgt ccc atg aca tct agt ctc ccg atc agt aca tgg aca ccg tct ttt				1022
Arg Pro Met Thr Ser Ser Leu Pro Ile Ser Thr Trp Thr Pro Ser Phe	305	310	315	
cct tct gat tca cga cac aag cat tct cat gac ttt aat atc act ttg				1070
Pro Ser Asp Ser Arg His Lys His Ser His Asp Phe Asn Ile Thr Leu	320	325	330	
acg tga tttcttctt gaagaactcg tagatcctct gtattttggt ttccagttta				1126
Thr				
gggctctaca tggttagactc tcaaagtcta ggtgttatgt tggctctgtca cttaggattg				1186
tcacttagga ttgtagacc atctccatca atggtttctc attgagaaac tgttcaatat				1246
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## MBI-20 Sequence Listing.ST25

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 Pro Arg Ser Glu Thr Ser Asn Thr Pro Pro Asn Ser Val Ala Pro Pro  
 35 40 45  
 Pro Pro Pro Pro Pro Gln Asn Ser Phe Thr Pro Ser Ala Ala Met Asp  
 50 55 60  
 Gly Phe Ser Ser Gly Pro Ile Lys Lys Arg Arg Gly Arg Pro Arg Lys  
 65 70 75 80  
 Tyr Gly His Asp Gly Ala Ala Val Thr Leu Ser Pro Asn Pro Ile Ser  
 85 90 95  
 Ser Ala Ala Pro Thr Thr Ser His Val Ile Asp Phe Ser Thr Thr Ser  
 100 105 110  
 Glu Lys Arg Gly Lys Met Lys Pro Ala Thr Pro Thr Pro Ser Ser Phe  
 115 120 125  
 Ile Arg Pro Lys Tyr Gln Val Glu Asn Leu Gly Glu Trp Ser Pro Ser  
 130 135 140  
 Ser Ala Ala Ala Asn Phe Thr Pro His Ile Ile Thr Val Asn Ala Gly  
 145 150 155 160  
 Glu Asp Val Thr Lys Arg Ile Ile Ser Phe Ser Gln Gln Gly Ser Leu  
 165 170 175  
 Ala Ile Cys Val Leu Cys Ala Asn Gly Val Val Ser Ser Val Thr Leu  
 180 185 190  
 Arg Gln Pro Asp Ser Ser Gly Gly Thr Leu Thr Tyr Glu Gly Arg Phe  
 195 200 205  
 Glu Ile Leu Ser Leu Ser Gly Thr Phe Met Pro Ser Asp Ser Asp Gly  
 210 215 220  
 Thr Arg Ser Arg Thr Gly Gly Met Ser Val Ser Leu Ala Ser Pro Asp  
 225 230 235 240  
 Gly Arg Val Val Gly Gly Gly Val Ala Gly Leu Leu Val Ala Ala Thr  
 245 250 255  
 Pro Ile Gln Val Val Val Gly Thr Phe Leu Gly Gly Thr Asn Gln Gln  
 260 265 270  
 Glu Gln Thr Pro Lys Pro His Asn His Asn Phe Met Ser Ser Pro Leu  
 275 280 285  
 Met Pro Thr Ser Ser Asn Val Ala Asp His Arg Thr Ile Arg Pro Met  
 290 295 300  
 Thr Ser Ser Leu Pro Ile Ser Thr Trp Thr Pro Ser Phe Pro Ser Asp  
 305 310 315 320

## MBI-20 Sequence Listing.ST25

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Asp Gln Pro Met Lys Pro Lys Thr Cys Ser Glu Ser Asp Phe Ala Asp  
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gat: tcc tct gct tct tct tct tct tct tgc gga caa aat ctc aga gga 152  
Asp Ser Ser Ala Ser Ser Ser Ser Ser Ser Gly Gln Asn Leu Arg Gly  
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gct: gag atg gtg gtg gaa gtg aag aag gaa gca gtt tgt tcc cag aaa 200  
Ala Glu Met Val Val Glu Val Lys Lys Glu Ala Val Cys Ser Gln Lys  
35 40 45  
gca: gag cga gag aag ctt cgt aga gat aag ctt aag gaa cag ttt ctt 248  
Ala Glu Arg Glu Lys Leu Arg Arg Asp Lys Leu Lys Glu Gln Phe Leu  
50 55 60 65  
gag: ctt gga aat gca ctt gat ccg aat agg cct aag agt gac aaa gcc 296  
Glu Leu Gly Asn Ala Leu Asp Pro Asn Arg Pro Lys Ser Asp Lys Ala  
70 75 80  
tca gtt ctc act gat aca ata caa atg ctc aag gat gta atg aac caa 344  
Ser Val Leu Thr Asp Thr Ile Gln Met Leu Lys Asp Val Met Asn Gln  
85 90 95  
gtt: gat aga cta aaa gct gag tat gaa aca cta tct caa gag tct cgt 392  
Val Asp Arg Leu Lys Ala Glu Tyr Glu Thr Leu Ser Gln Glu Ser Arg  
100 105 110  
gag: cta att caa gag aag agt gag ctg aga gag gag aaa gcg act tta 440  
Glu Leu Ile Gln Glu Lys Ser Glu Leu Arg Glu Lys Ala Thr Leu  
115 120 125  
aag: tct gat atc gag att ctt aat gct caa tat cag cat aga atc aaa 488  
Lys Ser Asp Ile Glu Ile Leu Asn Ala Gln Tyr Gln His Arg Ile Lys  
130 135 140 145  
acc: atg gtt cca tgg gta cct cat tac agt tat cat atc ccc ttc gta 536  
Thr Met Val Pro Trp Val Pro His Tyr Ser Tyr His Ile Pro Phe Val  
150 155 160  
gcc: ata act cag ggt cag tcc agt ttt ata cct tat tca gcc tct gtc 584  
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165 170 175  
aat: cct cta acc gaa caa caa gca tgc gtt cag cag cat tct tct tct 632  
Asn Pro Leu Thr Glu Gln Gln Ala Ser Val Gln Gln His Ser Ser Ser  
180 185 190  
tct: gcc gat gct tca atg aaa caa gat tcc aaa atc aag ccg tta gat 680  
Ser Ala Asp Ala Ser Met Lys Gln Asp Ser Lys Ile Lys Pro Leu Asp  
195 200 205  
ttg: gat ctg atg atg aac agt aac cat tca ggt caa gga aat gat caa 728

MBI-20 Sequence Listing.ST25

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210 215 220 225	
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Lys Asp Asp Val Arg Leu Lys Leu Glu Leu Lys Ile His Ala Ser Ser	
230 235 240	
tta gct caa cag gat gtt tct gga aaa gag aag aaa gta agc ttg aca	824
Leu Ala Gln Gln Asp Val Ser Gly Lys Glu Lys Lys Val Ser Leu Thr	
245 250 255	
acc act gca agc tca tcg aat agt tac tca tta tct caa gct gtt caa	872
Thr Thr Ala Ser Ser Ser Asn Ser Tyr Ser Leu Ser Gln Ala Val Gln	
260 265 270	
gat agt tcc ccc ggt acc gta aat gac atg ttg aag cca taa	914
Asp Ser Ser Pro Gly Thr Val Asn Asp Met Leu Lys Pro	
275 280 285	
accaataaac atattcccct gaacttggtg ttaataccgt gattgagaag gtaccatgat	974
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Lys Ala Glu Arg Glu Lys Leu Arg Arg Asp Lys Leu Lys Glu Gln Phe	
50 55 60	
Leu Glu Leu Gly Asn Ala Leu Asp Pro Asn Arg Pro Lys Ser Asp Lys	
65 70 75 80	
Ala Ser Val Leu Thr Asp Thr Ile Gln Met Leu Lys Asp Val Met Asn	
85 90 95	
Gln Val Asp Arg Leu Lys Ala Glu Tyr Glu Thr Leu Ser Gln Glu Ser	
100 105 110	
Arg Glu Leu Ile Gln Glu Lys Ser Glu Leu Arg Glu Glu Lys Ala Thr	
115 120 125	
Leu Lys Ser Asp Ile Glu Ile Leu Asn Ala Gln Tyr Gln His Arg Ile	
130 135 140	
Lys Thr Met Val Pro Trp Val Pro His Tyr Ser Tyr His Ile Pro Phe	
145 150 155 160	
Val Ala Ile Thr Gln Gly Gln Ser Ser Phe Ile Pro Tyr Ser Ala Ser	
165 170 175	

## MBI-20 Sequence Listing.ST25

Val. Asn Pro Leu Thr Glu Gln Gln Ala Ser Val Gln Gln His Ser Ser  
180 185 190

Ser Ser Ala Asp Ala Ser Met Lys Gln Asp Ser Lys Ile Lys Pro Leu  
195 200 205

Asp Leu Asp Leu Met Met Asn Ser Asn His Ser Gly Gln Gly Asn Asp  
210 215 220

Gln Lys Asp Asp Val Arg Leu Lys Leu Glu Leu Lys Ile His Ala Ser  
225 230 235 240

Ser Leu Ala Gln Gln Asp Val Ser Gly Lys Glu Lys Lys Val Ser Leu  
245 250 255

Thr Thr Thr Ala Ser Ser Ser Asn Ser Tyr Ser Leu Ser Gln Ala Val  
260 265 270

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ctccgatttc atcatcatct tccccatcat cgctcgtcttt gaaatcttgt cttctcaacg 180  
ctcttcactt ctgctgtaat aagcagaggg ttgttctgga gactccttct ctttccatgc 240  
gcttaagacc caaaaggact tgttctagtg ttgaagtctt tgggggtttt cacataaagc 300  
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agc gag att aag aag aga gct aag aga aac act cta tcg tcc ctt cct 517  
Ser Glu Ile Lys Lys Arg Ala Lys Arg Asn Thr Leu Ser Ser Leu Pro  
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Gln Glu Thr Gln Pro Leu Arg Lys Val Arg Ile Ile Val Asn Asp Pro  
35 40 45  
tat gct act gat gat tcc tct agt gat gag gaa gag ctt aag gtt cct 613  
Tyr Ala Thr Asp Asp Ser Ser Ser Asp Glu Glu Glu Leu Lys Val Pro  
50 55 60  
aag cca agg aaa atg aaa cgt atc gtt cgt gag att aac ttt cct tct 661  
Lys Pro Arg Lys Met Lys Arg Ile Val Arg Glu Ile Asn Phe Pro Ser  
65 70 75

## MBI-20 Sequence Listing.ST25

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atg gaa gtt tct gaa cag cct tct gag agt tct tct cag gac agt act 709
Met Glu Val Ser Glu Gln Pro Ser Glu Ser Ser Ser Gln Asp Ser Thr
80 85 90

aaa act gat ggc aag ata gct gtg tca gct tct cct gct gtt cct agg 757
Lys Thr Asp Gly Lys Ile Ala Val Ser Ala Ser Pro Ala Val Pro Arg
95 100 105 110

aag aag cct gtt ggt gtt agg caa agg aaa tgg ggg aaa tgg gct gct 805
Lys Lys Pro Val Gly Val Arg Gln Arg Lys Trp Gly Lys Trp Ala Ala
115 120 125

gag att aga gat cct att aag aaa act agg act tgg ttg ggt act ttt 853
Glu Ile Arg Asp Pro Ile Lys Lys Thr Arg Thr Trp Leu Gly Thr Phe
130 135 140

gat act ctt gaa gaa gct gct aaa gct tat gat gct aag aag ctt gag 901
Asp Thr Leu Glu Glu Ala Ala Lys Ala Tyr Asp Ala Lys Lys Leu Glu
145 150 155

ttt gat gct att gtt gct gga aat gtg tcc act act aaa cgt gat gtt 949
Phe Asp Ala Ile Val Ala Gly Asn Val Ser Thr Thr Lys Arg Asp Val
160 165 170

tct tca tct gag act agc caa tgc tct cgt tct tca cct gtt gtt cct 997
Ser Ser Ser Glu Thr Ser Gln Cys Ser Arg Ser Ser Pro Val Val Pro
175 180 185 190

gtt gag caa gat gac act tct gca tca gct ctc act tgt gtc aac aac 1045
Val Glu Gln Asp Asp Thr Ser Ala Ser Ala Leu Thr Cys Val Asn Asn
195 200 205

cct gat gac gtc tcg acc gtt gct cca act gct cca act cca aat gtt 1093
Pro Asp Asp Val Ser Thr Val Ala Pro Thr Ala Pro Thr Pro Asn Val
210 215 220

cct gct ggt gga aac aag gaa acg ttg ttc gat ttc gac ttt act aat 1141
Pro Ala Gly Gly Asn Lys Glu Thr Leu Phe Asp Phe Asp Phe Thr Asn
225 230 235

cta cag atc cct gat ttt ggt ttc ttg gca gag gag caa caa gac cta 1189
Leu Gln Ile Pro Asp Phe Gly Phe Leu Ala Glu Glu Gln Gln Asp Leu
240 245 250

gac ttc gat tgt ttc ctc gcg gat gat cag ttt gat gat ttc ggc ttg 1237
Asp Phe Asp Cys Phe Leu Ala Asp Asp Gln Phe Asp Asp Phe Gly Leu
255 260 265 270

ctt gat gac att caa gga ttc gaa gat aac ggt cca agt gcg tta cca 1285
Leu Asp Asp Ile Gln Gly Phe Glu Asp Asn Gly Pro Ser Ala Leu Pro
275 280 285

gat ttc gac ttt gcg gat gtt gaa gat ctt cag cta gct gac tct agt 1333
Asp Phe Asp Phe Ala Asp Val Glu Asp Leu Gln Leu Ala Asp Ser Ser
290 295 300

ttc ggt ttc ctt gat caa ctt gct cct atc aac atc tct tgc cca tta 1381
Phe Gly Phe Leu Asp Gln Leu Ala Pro Ile Asn Ile Ser Cys Pro Leu
305 310 315

aaa agt ttt gca gct tca tag gatccttgctt agtaatgtta agtgagaaga 1432
Lys Ser Phe Ala Ala Ser
320

gtgtttttgtt ttttcgttta tgcttttagta atttaagaca tacaaaagtg tgtgttccgg 1492

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## MBI-20 Sequence Listing.ST25

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Thr Gln Pro Leu Arg Lys Val Arg Ile Ile Val Asn Asp Pro Tyr Ala  
35 40 45

Thr Asp Asp Ser Ser Ser Asp Glu Glu Glu Leu Lys Val Pro Lys Pro  
50 55 60

Arg Lys Met Lys Arg Ile Val Arg Glu Ile Asn Phe Pro Ser Met Glu  
65 70 75 80

Val Ser Glu Gln Pro Ser Glu Ser Ser Ser Gln Asp Ser Thr Lys Thr  
85 90 95

Asp Gly Lys Ile Ala Val Ser Ala Ser Pro Ala Val Pro Arg Lys Lys  
100 105 110

Pro Val Gly Val Arg Gln Arg Lys Trp Gly Lys Trp Ala Ala Glu Ile  
115 120 125

Arg Asp Pro Ile Lys Lys Thr Arg Thr Trp Leu Gly Thr Phe Asp Thr  
130 135 140

Leu Glu Glu Ala Ala Lys Ala Tyr Asp Ala Lys Lys Leu Glu Phe Asp  
145 150 155 160

Ala Ile Val Ala Gly Asn Val Ser Thr Thr Lys Arg Asp Val Ser Ser  
165 170 175

Ser Glu Thr Ser Gln Cys Ser Arg Ser Ser Pro Val Val Pro Val Glu  
180 185 190

Gln Asp Asp Thr Ser Ala Ser Ala Leu Thr Cys Val Asn Asn Pro Asp  
195 200 205

Asp Val Ser Thr Val Ala Pro Thr Ala Pro Thr Pro Asn Val Pro Ala  
210 215 220

Gly Gly Asn Lys Glu Thr Leu Phe Asp Phe Asp Phe Thr Asn Leu Gln  
225 230 235 240

Ile Pro Asp Phe Gly Phe Leu Ala Glu Glu Gln Gln Asp Leu Asp Phe  
245 250 255

Asp Cys Phe Leu Ala Asp Asp Gln Phe Asp Asp Phe Gly Leu Leu Asp  
260 265 270

Asp Ile Gln Gly Phe Glu Asp Asn Gly Pro Ser Ala Leu Pro Asp Phe  
275 280 285

## MBI-20 Sequence Listing.ST25

Asp Phe Ala Asp Val Glu Asp Leu Gln Leu Ala Asp Ser Ser Phe Gly  
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Phe Leu Asp Gln Leu Ala Pro Ile Asn Ile Ser Cys Pro Leu Lys Ser  
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Phe Ala Ala Ser

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 <223> G1133

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 acc aaa cag cag aaa tgg ttg cca tta ggc tta aat cct caa gct tgt 163  
 Thr Lys Gln Gln Lys Trp Leu Pro Leu Gly Leu Asn Pro Gln Ala Cys  
 5 10 15 20  
 gtc cag gac aag gcg act gag tat ttc cgt cct gga att cct ttt ccg 211  
 Val Gln Asp Lys Ala Thr Glu Tyr Phe Arg Pro Gly Ile Pro Phe Pro  
 25 30 35  
 gaa ctc ggt aaa gtt tat gca gct gag cat cag ttt cgc tat ttg cag 259  
 Glu Leu Gly Lys Val Tyr Ala Ala Glu His Gln Phe Arg Tyr Leu Gln  
 40 45 50  
 cca ccg ttc caa gcc tta ttg tct aga tat gat cag cag tct tgt gga 307  
 Pro Pro Phe Gln Ala Leu Leu Ser Arg Tyr Asp Gln Gln Ser Cys Gly  
 55 60 65  
 aaa caa gtt tca tgt ttg aat ggg cga tct agc aac ggt gct gct cca 355  
 Lys Gln Val Ser Cys Leu Asn Gly Arg Ser Ser Asn Gly Ala Ala Pro  
 70 75 80  
 gag ggg gca ctc aag tct tct cgg aaa aga ttt ata gta ttc gat cag 403  
 Glu Gly Ala Leu Lys Ser Ser Arg Lys Arg Phe Ile Val Phe Asp Gln  
 85 90 95 100  
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 Ser Gly Glu Gln Thr Arg Leu Leu Gln Cys Gly Phe Pro Leu Arg Phe  
 105 110 115  
 cct tct tct atg gat gca gag cga ggg aac att ctc ggt gcc cta cac 499  
 Pro Ser Ser Met Asp Ala Glu Arg Gly Asn Ile Leu Gly Ala Leu His  
 120 125 130  
 cca gag aaa ggg ttt agt aaa gat cat gcc att caa gaa aag ata ttg 547  
 Pro Glu Lys Gly Phe Ser Lys Asp His Ala Ile Gln Glu Lys Ile Leu  
 135 140 145  
 caa cat gaa gat cat gaa aat ggc gaa gaa gac tcg gaa atg cac gaa 595  
 Gln His Glu Asp His Glu Asn Gly Glu Glu Asp Ser Glu Met His Glu  
 150 155 160  
 gac act gag gaa atc aac gcg tta ctg tat tct gat gat gac gat aat 643  
 Asp Thr Glu Glu Ile Asn Ala Leu Leu Tyr Ser Asp Asp Asp Asp Asn  
 165 170 175 180

## MBI-20 Sequence Listing.ST25

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 Phe Thr Val Glu Gln Gln Ala Cys Asn Ile Thr Thr Glu Glu Leu Asp  
 200 205 210  
 gaa act gaa agc act gtt gat ggt cca ctt ctt aaa aga cag aaa cta 787  
 Glu Thr Glu Ser Thr Val Asp Gly Pro Leu Leu Lys Arg Gln Lys Leu  
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 ctg gac cat tcg tac aga gac tca tca cca tcc ctt gtg ggc acc act 835  
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 230 235 240  
 aaa gtc aaa ggc tta tca gat gaa aac ctt cct gaa tca aac att tca 883  
 Lys Val Lys Gly Leu Ser Asp Glu Asn Leu Pro Glu Ser Asn Ile Ser  
 245 250 255 260  
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 Ser Lys Gln Glu Thr Gly Ser Gly Leu Ser Asp Glu Gln Ser Arg Lys  
 265 270 275  
 gac aag att cac acc gct ctg aga atc ctg gag agt gta gtt cca ggg 979  
 Asp Lys Ile His Thr Ala Leu Arg Ile Leu Glu Ser Val Val Pro Gly  
 280 285 290  
 gca aag gga aaa gaa gct ctt tta cta cta gac gaa gcc att gat tac 1027  
 Ala Lys Gly Lys Glu Ala Leu Leu Leu Leu Asp Glu Ala Ile Asp Tyr  
 295 300 305  
 ctg aag ttg ctg aag caa agc tta aac tca tca aag ggt ttg aat aac 1075  
 Leu Lys Leu Leu Lys Gln Ser Leu Asn Ser Ser Lys Gly Leu Asn Asn  
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 His Trp  
 325  
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 gaagattctc tcaaatcat taacgtgggt ttgaaacaat tagaacacgc ctggtgaccc 1244  
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 35 40 45  
 Arg Tyr Leu Gln Pro Pro Phe Gln Ala Leu Leu Ser Arg Tyr Asp Gln  
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 Gln Ser Cys Gly Lys Lys Gln Val Ser Cys Leu Asn Gly Arg Ser Ser Asn  
 65 70 75 80



## MBI-20 Sequence Listing.ST25

Gly Ala Ala Pro Glu Gly Ala Leu Lys Ser Ser Arg Lys Arg Phe Ile  
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                   100                  105                  110  
 Pro Leu Arg Phe Pro Ser Ser Met Asp Ala Glu Arg Gly Asn Ile Leu  
                   115                  120                  125  
 Gly Ala Leu His Pro Glu Lys Gly Phe Ser Lys Asp His Ala Ile Gln  
                   130                  135                  140  
 Glu Lys Ile Leu Gln His Glu Asp His Glu Asn Gly Glu Glu Asp Ser  
                   145                  150                  155                  160  
 Glu Met His Glu Asp Thr Glu Glu Ile Asn Ala Leu Leu Tyr Ser Asp  
                   165                  170                  175  
 Asp Asp Asp Asn Asp Asp Trp Glu Ser Asp Asp Glu Val Met Ser Thr  
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 Gly His Ser Pro Phe Thr Val Glu Gln Gln Ala Cys Asn Ile Thr Thr  
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 Glu Glu Leu Asp Glu Thr Glu Ser Thr Val Asp Gly Pro Leu Leu Lys  
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 Arg Gln Lys Leu Leu Asp His Ser Tyr Arg Asp Ser Ser Pro Ser Leu  
                   225                  230                  235                  240  
 Val Gly Thr Thr Lys Val Lys Gly Leu Ser Asp Glu Asn Leu Pro Glu  
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 Ser Asn Ile Ser Ser Lys Gln Glu Thr Gly Ser Gly Leu Ser Asp Glu  
                   260                  265                  270  
 Gln Ser Arg Lys Asp Lys Ile His Thr Ala Leu Arg Ile Leu Glu Ser  
                   275                  280                  285  
 Val Val Pro Gly Ala Lys Gly Lys Glu Ala Leu Leu Leu Asp Glu  
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 Ala Ile Asp Tyr Leu Lys Leu Leu Lys Gln Ser Leu Asn Ser Ser Lys  
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 Gly Leu Asn Asn His Trp  
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 tat tct atc gga tct tct cca gat tct ttc tca tcc tct tct tct aac 157  
 Tyr Ser Ile Gly Ser Ser Pro Asp Ser Phe Ser Ser Ser Ser Ser Asn  
 20 25 30  
 aat tac tct ctt ccc ttc aac gag aac gac tca gag gaa atg ttt ctc 205  
 Asn Tyr Ser Leu Pro Phe Asn Glu Asn Asp Ser Glu Glu Met Phe Leu  
 35 40 45  
 tac ggt cta atc gag cag tcc acg caa caa acc tat att gac tcg gat 253  
 Tyr Gly Leu Ile Glu Gln Ser Thr Gln Gln Thr Tyr Ile Asp Ser Asp  
 50 55 60  
 agt caa gac ctt ccg atc aaa tcc gta agc tca aga aag tca gag aag 301  
 Ser Gln Asp Leu Pro Ile Lys Ser Val Ser Ser Arg Lys Ser Glu Lys  
 65 70 75 80  
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 Ser Tyr Arg Gly Val Arg Arg Arg Pro Trp Gly Lys Phe Ala Ala Glu  
 85 90 95  
 ata aga gat tcg act aga aac ggt att agg gtt tgg ctc ggg acg ttc 397  
 Ile Arg Asp Ser Thr Arg Asn Gly Ile Arg Val Trp Leu Gly Thr Phe  
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 gaa agc gcg gaa gag gcg gct tta gcc tac gat caa gct gct ttc tcg 445  
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 115 120 125  
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 Met Arg Gly Ser Ser Ala Ile Leu Asn Phe Ser Ala Glu Arg Val Gln  
 130 135 140  
 gag tcg ctt tcg gag att aaa tat acc tac gag gat ggt tgt tct ccg 541  
 Glu Ser Leu Ser Glu Ile Lys Tyr Thr Tyr 155 Asp Gly Cys Ser Pro  
 145 150 155 160  
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 165 170 175  
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 Lys Lys Thr Lys Asp Ser Asp Phe Asp His Arg Ser Val Lys Leu Asp  
 180 185 190  
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Asn	Tyr	Ser 35	Leu	Pro	Phe	Asn	Glu 40	Asn	Asp	Ser	Glu	Glu 45	Met	Phe	Leu
Tyr	Gly 50	Leu	Ile	Glu	Gln	Ser 55	Thr	Gln	Gln	Thr	Tyr 60	Ile	Asp	Ser	Asp
Ser 65	Gln	Asp	Leu	Pro	Ile 70	Lys	Ser	Val	Ser	Ser 75	Arg	Lys	Ser	Glu	Lys 80
Ser	Tyr	Arg	Gly	Val 85	Arg	Arg	Arg	Pro	Trp 90	Gly	Lys	Phe	Ala	Ala 95	Glu
Ile	Arg	Asp	Ser 100	Thr	Arg	Asn	Gly	Ile 105	Arg	Val	Trp	Leu	Gly 110	Thr	Phe
Glu	Ser	Ala 115	Glu	Glu	Ala	Ala	Leu 120	Ala	Tyr	Asp	Gln	Ala 125	Ala	Phe	Ser
Met	Arg 130	Gly	Ser	Ser	Ala	Ile 135	Leu	Asn	Phe	Ser	Ala 140	Glu	Arg	Val	Gln
Glu 145	Ser	Leu	Ser	Glu	Ile 150	Lys	Tyr	Thr	Tyr	Glu 155	Asp	Gly	Cys	Ser	Pro 160
Val	Val	Ala	Leu	Lys 165	Arg	Lys	His	Ser	Met 170	Arg	Arg	Arg	Met	Thr 175	Asn
Lys	Lys	Thr 180	Lys	Asp	Ser	Asp	Phe	Asp 185	His	Arg	Ser	Val	Lys 190	Leu	Asp
Asn	Val	Val 195	Val	Phe	Glu	Asp	Leu 200	Gly	Glu	Gln	Tyr	Leu 205	Glu	Glu	Leu
Leu	Gly 210	Ser	Ser	Glu	Asn	Ser 215	Gly	Thr	Trp						

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<223> G1324
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Asn Tyr Ile Leu His Asn Gly Glu Gly Arg Trp Asn His Val Ala Lys																	
35 40 45																	
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Cys Ala Gly Leu Lys Arg Thr Gly Lys Ser Cys Arg Leu Arg Trp Leu																	
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Asn Tyr Leu Lys Pro Asp Ile Arg Arg Gly Asn Leu Thr Pro Gln Glu																	
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85 90 95																	
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Lys Ile Ala Gln Tyr Leu Pro Gly Arg Thr Asp Asn Glu Ile Lys Asn																	
100 105 110																	
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115 120 125																	
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Arg Leu Ile Glu Lys Met Glu Gln Asn Ser Ser Thr Thr Thr Thr Tyr																	
150 155 160																	
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Cys Cys Pro Gln Asn Asn Asn Asn Asn Ser Leu Leu Leu Pro Ser Gln																	
165 170 175																	
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Ser His Asp Ser Leu Ser Met Gln Lys Asp Ile Asp Tyr Ser Gly Phe																	
180 185 190																	
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Ser Asn Ile Asp Gly Ser Ser Ser Thr Ser Thr Cys Met Ser His Leu																	
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Thr Thr Val Pro His Phe Met Asp Gln Ser Asn Thr Asn Ile Ile Asp																	
210 215 220 225																	
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Gly Ser Met Cys Phe His Glu Gly Asn Val Gln Glu Phe Gly Gly Tyr																	
230 235 240																	
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Val Pro Gly Met Glu Asp Tyr Met Val Asn Ser Asp Ile Ser Met Glu																	
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Cys His Val Ala Asp Gly Tyr Ser Ala Tyr Glu Asp Val Thr Gln Asp																	
260 265 270																	
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275 280 285																	
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## MBI-20 Sequence Listing.ST25

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 35 40 45  
 Lys Cys Ala Gly Leu Lys Arg Thr Gly Lys Ser Cys Arg Leu Arg Trp  
 50 55 60  
 Leu Asn Tyr Leu Lys Pro Asp Ile Arg Arg Gly Asn Leu Thr Pro Gln  
 65 70 75 80  
 Glu Gln Leu Leu Ile Leu Glu Leu His Ser Lys Trp Gly Asn Arg Trp  
 85 90 95  
 Ser Lys Ile Ala Gln Tyr Leu Pro Gly Arg Thr Asp Asn Glu Ile Lys  
 100 105 110  
 Asn Tyr Trp Arg Thr Arg Val Gln Lys Gln Ala Arg Gln Leu Asn Ile  
 115 120 125  
 Glu Ser Asn Ser Asp Lys Phe Phe Asp Ala Val Arg Ser Phe Trp Val  
 130 135 140  
 Pro Arg Leu Ile Glu Lys Met Glu Gln Asn Ser Ser Thr Thr Thr Thr  
 145 150 155 160  
 Tyr Cys Cys Pro Gln Asn Asn Asn Asn Asn Ser Leu Leu Leu Pro Ser  
 165 170 175  
 Gln Ser His Asp Ser Leu Ser Met Gln Lys Asp Ile Asp Tyr Ser Gly  
 180 185 190  
 Phe Ser Asn Ile Asp Gly Ser Ser Ser Thr Ser Thr Cys Met Ser His  
 195 200 205  
 Leu Thr Thr Val Pro His Phe Met Asp Gln Ser Asn Thr Asn Ile Ile  
 210 215 220  
 Asp Gly Ser Met Cys Phe His Glu Gly Asn Val Gln Glu Phe Gly Gly  
 225 230 235 240  
 Tyr Val Pro Gly Met Glu Asp Tyr Met Val Asn Ser Asp Ile Ser Met  
 245 250 255  
 Glu Cys His Val Ala Asp Gly Tyr Ser Ala Tyr Glu Asp Val Thr Gln  
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## MBI-20 Sequence Listing.ST25

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 Met Ser Ser Ser Glu Arg  
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gta ccg tgc gat ttc tgc ggc gag cgt acg gcg gtt ttg ttt tgt aga 162  
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 Ala Asp Thr Ala Lys Leu Cys Leu Pro Cys Asp Gln Gln Val His Thr  
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 Ala Asn Leu Leu Ser Arg Lys His Val Arg Ser Gln Ile Cys Asp Asn  
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tgc ggt aac gag cca gtc tct gtt cgg tgt ttc acc gat aat ctg att 306  
 Cys Gly Asn Glu Pro Val Ser Val Arg Cys Phe Thr Asp Asn Leu Ile  
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 75 80 85

gat gct cat gtt cga tcc gcc gtg gaa ggt ttt tcc ggt tgt cca tcg 402  
 Asp Ala His Val Arg Ser Ala Val Glu Gly Phe Ser Gly Cys Pro Ser  
 90 95 100

gcg ttg gag ctt gct gct tta tgg gga ctt gat ttg gag caa ggg agg 450  
 Ala Leu Glu Leu Ala Ala Leu Trp Gly Leu Asp Leu Glu Gln Gly Arg  
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 Lys Asp Glu Glu Asn Gln Val Pro Met Met Ala Met Met Met Asp Asn  
 120 125 130

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 Phe Gly Met Gln Leu Asp Ser Trp Val Leu Gly Ser Asn Glu Leu Ile  
 135 140 145 150

gtt ccc agc gat acg acg ttt aag aag cgt gga tct tgt gga tct agt 594  
 Val Pro Ser Asp Thr Thr Phe Lys Lys Arg Gly Ser Cys Gly Ser Ser  
 155 160 165

tgc ggg agg tat aag cag gta ttg tgt aag cag ctt gag gag ttg ctt 642  
 Cys Gly Arg Tyr Lys Gln Val Leu Cys Lys Gln Leu Glu Glu Leu Leu  
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aag agt ggt gtt gtc ggt ggt gat ggc gat gat ggt gat cgt gac cgt 690  
 Lys Ser Gly Val Val Gly Gly Asp Gly Asp Asp Gly Asp Arg Asp Arg  
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gat tgt gac cgt gag ggt gct tgt gat gga gat gga gat gga gaa gca 738  
 Asp Cys Asp Arg Glu Gly Ala Cys Asp Gly Asp Gly Asp Gly Glu Ala  
 200 205 210

## MBI-20 Sequence Listing.ST25

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gga gag ggg ctt atg gtt ccg gag atg tca gag aga ttg aaa tgg tca      786
Gly Glu Gly Leu Met Val Pro Glu Met Ser Glu Arg Leu Lys Trp Ser
215                220                225                230

aga gat gtt gag gag atc aat ggt ggc gga gga gga gga gtt aac cag      834
Arg Asp Val Glu Glu Ile Asn Gly Gly Gly Gly Gly Gly Val Asn Gln
                235                240                245

cag tgg aat gct act act act aat cct agt ggt ggc cag agt tct cag      882
Gln Trp Asn Ala Thr Thr Thr Asn Pro Ser Gly Gly Gln Ser Ser Gln
                250                255                260

ata tgg gat ttt aac ttg gga cag tca cgg gga cct gag gat acg agt      930
Ile Trp Asp Phe Asn Leu Gly Gln Ser Arg Gly Pro Glu Asp Thr Ser
                265                270                275

cga gtg gaa gct gca tat gta ggg aaa ggt gct gct tct tca ttc aca      978
Arg Val Glu Ala Ala Tyr Val Gly Lys Gly Ala Ala Ser Ser Phe Thr
280                285                290

atc aac aat ttt gtt gac cat atg aat gaa act tgt tcc act aat gtg      1026
Ile Asn Asn Phe Val Asp His Met Asn Glu Thr Cys Ser Thr Asn Val
295                300                305                310

aaa ggt gtc aaa gag att aaa aag gat gac tac aag cga tca act tca      1074
Lys Gly Val Lys Glu Ile Lys Lys Asp Asp Tyr Lys Arg Ser Thr Ser
315                320                325

ggc cag gta caa cca aca aaa tct gag agc aac aat cgt cca att acc      1122
Gly Gln Val Gln Pro Thr Lys Ser Glu Ser Asn Asn Arg Pro Ile Thr
330                335                340

ttt ggc tct gag aaa ggt tcg aac tcc tcc agt gac ttg cat ttc aca      1170
Phe Gly Ser Glu Lys Gly Ser Asn Ser Ser Ser Asp Leu His Phe Thr
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gag cat att gct gga act agt tgt aag acc aca aga cta gtt gca act      1218
Glu His Ile Ala Gly Thr Ser Cys Lys Thr Thr Arg Leu Val Ala Thr
360                365                370

aag gct gat ctg gag cgg ctg gct cag aac aga gga gat gca atg cag      1266
Lys Ala Asp Leu Glu Arg Leu Ala Gln Asn Arg Gly Asp Ala Met Gln
375                380                385                390

cgt tac aag gaa aag agg aag aca cgg aga tat gat aag acc ata agg      1314
Arg Tyr Lys Glu Lys Arg Lys Thr Arg Arg Tyr Asp Lys Thr Ile Arg
395                400                405

tat gaa tcg agg aag gca aga gct gac act agg ttg cgt gtc aga ggc      1362
Tyr Glu Ser Arg Lys Ala Arg Ala Asp Thr Arg Leu Arg Val Arg Gly
410                415                420

aga ttt gtg aaa gct agt gaa gct cct tac cct taa ccttaagttt      1408
Arg Phe Val Lys Ala Ser Glu Ala Pro Tyr Pro
425                430

tttcacatag gcttcctttt agctacaaac ttagttactt tttttactcc actgcctcat      1468

aaatgtacag accggtctcg tttcatctgg ccgccccttct tgttttattg ccttatctgg      1528

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Ser	Gln	Ile	Cys	Asp	Asn	Cys	Gly	Asn	Glu	Pro	Val	Ser	Val	Arg	Cys	
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Phe	Thr	Asp	Asn	Leu	Ile	Leu	Cys	Gln	Glu	Cys	Asp	Trp	Asp	Val	His	
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Gly	Ser	Cys	Ser	Val	Ser	Asp	Ala	His	Val	Arg	Ser	Ala	Val	Glu	Gly	
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Phe	Ser	Gly	Cys	Pro	Ser	Ala	Leu	Glu	Leu	Ala	Ala	Leu	Trp	Gly	Leu	
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Ala	Met	Met	Met	Asp	Asn	Phe	Gly	Met	Gln	Leu	Asp	Ser	Trp	Val	Leu	
	130					135					140					
Gly	Ser	Asn	Glu	Leu	Ile	Val	Pro	Ser	Asp	Thr	Thr	Phe	Lys	Lys	Arg	
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Gly	Ser	Cys	Gly	Ser	Ser	Cys	Gly	Arg	Tyr	Lys	Gln	Val	Leu	Cys	Lys	
				165					170					175		
Gln	Leu	Glu	Glu	Leu	Leu	Lys	Ser	Gly	Val	Val	Gly	Gly	Asp	Gly	Asp	
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Asp	Gly	Asp	Arg	Asp	Arg	Asp	Cys	Asp	Arg	Glu	Gly	Ala	Cys	Asp	Gly	
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Asp	Gly	Asp	Gly	Glu	Ala	Gly	Glu	Gly	Leu	Met	Val	Pro	Glu	Met	Ser	
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Glu	Arg	Leu	Lys	Trp	Ser	Arg	Asp	Val	Glu	Glu	Ile	Asn	Gly	Gly	Gly	
225					230					235					240	
Gly	Gly	Gly	Val	Asn	Gln	Gln	Trp	Asn	Ala	Thr	Thr	Thr	Asn	Pro	Ser	
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Gly	Gly	Gln	Ser	Ser	Gln	Ile	Trp	Asp	Phe	Asn	Leu	Gly	Gln	Ser	Arg	
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Gly	Pro	Glu	Asp	Thr	Ser	Arg	Val	Glu	Ala	Ala	Tyr	Val	Gly	Lys	Gly	
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Ala	Ala	Ser	Ser	Phe	Thr	Ile	Asn	Asn	Phe	Val	Asp	His	Met	Asn	Glu	
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Met	Val	Gln	Thr	Lys	Lys	Phe	Arg	Gly	Val	Arg	Gln	Arg	His	Trp	Gly		
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Ser	Trp	Val	Ala	Glu	Ile	Arg	His	Pro	Leu	Leu	Lys	Arg	Arg	Ile	Trp		
			20					25				30					
cta ggg acg ttc gag acc gca gag gag gca gca aga gca tac gac gag																	201
Leu	Gly	Thr	Phe	Glu	Thr	Ala	Glu	Glu	Ala	Ala	Arg	Ala	Tyr	Asp	Glu		
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gcc gcc gtt tta atg agc ggc cgc aac gcc aaa acc aac ttt ccc ctc																	249
Ala	Ala	Val	Leu	Met	Ser	Gly	Arg	Asn	Ala	Lys	Thr	Asn	Phe	Pro	Leu		
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aac aac aac aac acc gga gaa act tcc gag ggc aaa acc gat att tca																	297
Asn	Asn	Asn	Asn	Thr	Gly	Glu	Thr	Ser	Glu	Gly	Lys	Thr	Asp	Ile	Ser		
65					70					75				80			
gct tcg tcc aca atg tca tcc tca aca tca tct tca tcg ctc tct tcc																	345
Ala	Ser	Ser	Thr	Met	Ser	Ser	Ser	Thr	Ser	Ser	Ser	Ser	Leu	Ser	Ser		
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atc ctc agc gcc aaa ctg agg aaa tgc tgc aag tct cct tcc cca tcc																	393
Ile	Leu	Ser	Ala	Lys	Leu	Arg	Lys	Cys	Cys	Lys	Ser	Pro	Ser	Pro	Ser		

## MBI-20 Sequence Listing.ST25

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ctc acc tgc ctc cgt ctt gac aca gcc agc tcc cat atc ggc gtc tgg      441
Leu Thr Cys Leu Arg Leu Asp Thr Ala Ser Ser His Ile Gly Val Trp
      115      120      125

cag aaa cgg gcc ggt tca aag tct gac tcc agc tgg gtc atg acg gtg      489
Gln Lys Arg Ala Gly Ser Lys Ser Asp Ser Ser Trp Val Met Thr Val
      130      135      140

gag cta ggt ccc gca agc tcc tcc caa gag act act agt aaa gct tca      537
Glu Leu Gly Pro Ala Ser Ser Ser Gln Glu Thr Thr Ser Lys Ala Ser
      145      150      155      160

caa gac gct att ctt gct ccg acc act gaa gtt gaa att ggt ggc agc      585
Gln Asp Ala Ile Leu Ala Pro Thr Thr Glu Val Glu Ile Gly Gly Ser
      165      170      175

aga gaa gaa gta ttg gat gag gaa gaa aag gtt gct ttg caa atg ata      633
Arg Glu Glu Val Leu Asp Glu Glu Lys Val Ala Leu Gln Met Ile
      180      185      190

gag gag ctt ctc aat aca aac taa atcttatttg cttatatata tgtacctatt      687
Glu Glu Leu Leu Asn Thr Asn
      195

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Leu Gly Thr Phe Glu Thr Ala Glu Glu Ala Ala Arg Ala Tyr Asp Glu
35      40      45

Ala Ala Val Leu Met Ser Gly Arg Asn Ala Lys Thr Asn Phe Pro Leu
50      55      60

Asn Asn Asn Asn Thr Gly Glu Thr Ser Glu Gly Lys Thr Asp Ile Ser
65      70      75      80

Ala Ser Ser Thr Met Ser Ser Ser Thr Ser Ser Ser Ser Leu Ser Ser
85      90      95

Ile Leu Ser Ala Lys Leu Arg Lys Cys Cys Lys Ser Pro Ser Pro Ser
100      105      110

Leu Thr Cys Leu Arg Leu Asp Thr Ala Ser Ser His Ile Gly Val Trp
115      120      125

Gln Lys Arg Ala Gly Ser Lys Ser Asp Ser Ser Trp Val Met Thr Val
130      135      140

Glu Leu Gly Pro Ala Ser Ser Ser Gln Glu Thr Thr Ser Lys Ala Ser

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145 150 155 160

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## MBI-20 Sequence Listing.ST25

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Thr Ser Thr Gly Lys Glu Asn Gln Asp Glu Asn Cys Ser Gly Val Ser				
	155	160	165	
acc gtg aac aag tat ccc tta cca acg aaa cag gta agt ggc gac att				883
Thr Val Asn Lys Tyr Pro Leu Pro Thr Lys Gln Val Ser Gly Asp Ile				
	170	175	180	
gaa aca agt aag acc tca act gtg gac aac gcg gtt caa gat gtt ccc				931
Glu Thr Ser Lys Thr Ser Thr Val Asp Asn Ala Val Gln Asp Val Pro				
	185	190	195	
aag aag aac aaa gac aaa gat ggt aac gat ggt act act gtg cac agc				979
Lys Lys Asn Lys Asp Lys Asp Gly Asn Asp Gly Thr Thr Val His Ser				
	200	205	210	
atg caa aac tac cct tgg cat ttc cac gca gat att gtg aac ggg aat				1027
Met Gln Asn Tyr Pro Trp His Phe His Ala Asp Ile Val Asn Gly Asn				
	215	220	225	230
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Ile Ala Lys Cys Pro Gln Asn His Pro Ser Gly Met Val Ser Gln Asp				
	235	240	245	
ttc atg ttt cat cct atg aga gaa gaa act cac ggg cac gca aat ctt				1123
Phe Met Phe His Pro Met Arg Glu Glu Thr His Gly His Ala Asn Leu				
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caa gct aca aca gca tct gct act act aca gct tct cat caa gcg ttt				1171
Gln Ala Thr Thr Ala Ser Ala Thr Thr Thr Ala Ser His Gln Ala Phe				
	265	270	275	
cca gct tgt cat tca cag gat gat tac cgt tcg ttt ctc cag ata tca				1219
Pro Ala Cys His Ser Gln Asp Asp Tyr Arg Ser Phe Leu Gln Ile Ser				
	280	285	290	
tct act ttc tcc aat ctt att atg tca act ctc cta cag aat cct gca				1267
Ser Thr Phe Ser Asn Leu Ile Met Ser Thr Leu Leu Gln Asn Pro Ala				
	295	300	305	310
gct cat gct gca gct aca ttc gct gct tcg gtc tgg cct tat gcg agt				1315
Ala His Ala Ala Ala Thr Phe Ala Ala Ser Val Trp Pro Tyr Ala Ser				
	315	320	325	
gtc ggg aat tct ggt gat tca tca acc cca atg agc tct tct cct cca				1363
Val Gly Asn Ser Gly Asp Ser Ser Pro Met Ser Ser Ser Pro Pro				
	330	335	340	
agt ata act gcc att gcc gct gct aca gta gct gct gca act gct tgg				1411
Ser Ile Thr Ala Ile Ala Ala Thr Val Ala Ala Thr Ala Trp				
	345	350	355	
tgg gct tct cat gga ctt ctt cct gta tgc gct cca gct cca ata aca				1459
Trp Ala Ser His Gly Leu Leu Pro Val Cys Ala Pro Ala Pro Ile Thr				
	360	365	370	
tgt gtt cca ttc tca act gtt gca gtt cca act cca gca atg act gaa				1507
Cys Val Pro Phe Ser Thr Val Ala Val Pro Thr Pro Ala Met Thr Glu				
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atg gat acc gtt gaa aat act caa ccg ttt gag aaa caa aac aca gct				1555
Met Asp Thr Val Glu Asn Thr Gln Pro Phe Glu Lys Gln Asn Thr Ala				
	395	400	405	
ctg caa gat caa acc ttg gct tcg aaa tct cca gct tca tca tct gat				1603
Leu Gln Asp Gln Thr Leu Ala Ser Lys Ser Pro Ala Ser Ser Asp				
	410	415	420	
gat tca gat gag act gga gta acc aag cta aat gcc gac tca aaa acc				1651
Asp Ser Asp Glu Thr Gly Val Thr Lys Leu Asn Ala Asp Ser Lys Thr				
	425	430	435	
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MBI-20 Sequence Listing.ST25

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Ser	Asn	Thr	Ala	Gln	Lys	Lys	Asn	Leu	Val	Asp	Arg	Ser	Ser	Cys	Gly		
455					460					465					470		
tca	aat	aca	cct	tca	ggg	agt	gac	gca	gaa	act	gat	gca	tta	gat	aaa	1795	
Ser	Asn	Thr	Pro		Gly	Ser	Asp	Ala	Glu	Thr	Asp	Ala	Leu	Asp	Lys		
				475					480					485			
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Met	Glu	Lys	Asp	Lys	Glu	Asp	Val	Lys	Glu	Thr	Asp	Glu	Asn	Gln	Pro		
			490					495					500				
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Asp	Val	Ile	Glu	Leu	Asn	Asn	Arg	Lys	Ile	Lys	Met	Arg	Asp	Asn	Asn		
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Ser	Asn	Asn	Asn	Ala	Thr	Thr	Asp	Ser	Trp	Lys	Glu	Val	Ser	Glu	Glu		
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Gly	Arg	Ile	Ala	Phe	Gln	Ala	Leu	Phe	Ala	Arg	Glu	Arg	Leu	Pro	Gln		
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Ser	Phe	Ser	Pro	Pro	Gln	Val	Ala	Glu	Asn	Val	Asn	Arg	Lys	Gln	Ser		
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			600			605					610						
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## MBI-20 Sequence Listing.ST25

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 Ile Pro Val Cys Gln Ala Leu Asp Ile Glu Ile Pro Pro Pro Arg Pro  
 85 90 95  
 Lys Arg Lys Pro Asn Thr Pro Tyr Pro Arg Lys Pro Gly Asn Asn Gly  
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 Ser Ala Ser Ser Ser Gln Leu Asn Gln Ala Phe Leu Asp Leu Glu Lys  
 130 135 140  
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 Gly Thr Thr Val His Ser Met Gln Asn Tyr Pro Trp His Phe His Ala  
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 Gly Met Val Ser Gln Asp Phe Met Phe His Pro Met Arg Glu Glu Thr  
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 His Gly His Ala Asn Leu Gln Ala Thr Thr Ala Ser Ala Thr Thr Thr  
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## MBI-20 Sequence Listing.ST25

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405                               410                               415
Pro Ala Ser Ser Ser Asp Asp Ser Asp Glu Thr Gly Val Thr Lys Leu
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Asn Ala Asp Ser Lys Thr Asn Asp Asp Lys Ile Glu Glu Val Val Val
435                               440                               445
Thr Ala Ala Val His Asp Ser Asn Thr Ala Gln Lys Lys Asn Leu Val
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Asp Arg Ser Ser Cys Gly Ser Asn Thr Pro Ser Gly Ser Asp Ala Glu
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Thr Asp Ala Leu Asp Lys Met Glu Lys Asp Lys Glu Asp Val Lys Glu
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Lys Met Arg Asp Asn Asn Ser Asn Asn Asn Ala Thr Thr Asp Ser Trp
515                               520                               525
Lys Glu Val Ser Glu Glu Gly Arg Ile Ala Phe Gln Ala Leu Phe Ala
530                               535                               540
Arg Glu Arg Leu Pro Gln Ser Phe Ser Pro Pro Gln Val Ala Glu Asn
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Val Asn Arg Lys Gln Ser Asp Thr Ser Met Pro Leu Ala Pro Asn Phe
565                               570                               575
Lys Ser Gln Asp Ser Cys Ala Ala Asp Gln Glu Gly Val Val Met Ile
580                               585                               590
Gly Val Gly Thr Cys Lys Ser Leu Lys Thr Arg Gln Thr Gly Phe Lys
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## MBI-20 Sequence Listing.ST25

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 1 5 10  
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 Thr Ala Ile Gln Glu Ala Ala Ser Gln Gly Leu Gln Ser Met Glu His  
 15 20 25 30  
 ctg atc cgt gtc ctc tct aac cgt ccc gaa caa caa cac aac gtt gac 204  
 Leu Ile Arg Val Ser Asn Arg Pro Glu Gln Gln His Asn Val Asp  
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 Cys Ser Glu Ile Thr Asp Phe Thr Val Ser Lys Phe Lys Thr Val Ile  
 50 55 60  
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 Ser Leu Leu Asn Arg Thr Gly His Ala Arg Phe Arg Arg Gly Pro Val  
 65 70 75  
 cac tcc act tcc tct gcc gca tct cag aaa cta cag agt cag atc gtt 348  
 His Ser Thr Ser Ser Ala Ala Ser Gln Lys Leu Gln Ser Gln Ile Val  
 80 85 90  
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 Lys Asn Thr Gln Pro Glu Ala Pro Ile Val Arg Thr Thr Thr Asn His  
 95 100 105 110  
 cct caa atc gtt cct cca ccg tct agt gta aca ctc gat ttc tct aaa 444  
 Pro Gln Ile Val Pro Pro Ser Ser Val Thr Leu Asp Phe Ser Lys  
 115 120 125  
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 Pro Ser Ile Phe Gly Thr Lys Ala Lys Ser Ala Glu Leu Glu Phe Ser  
 130 135 140  
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 Lys Glu Asn Phe Ser Val Ser Leu Asn Ser Ser Phe Met Ser Ser Ala  
 145 150 155  
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 Ile Thr Gly Asp Gly Ser Val Ser Asn Gly Lys Ile Phe Leu Ala Ser  
 160 165 170  
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 Ala Pro Ser Gln Pro Val Asn Ser Ser Gly Lys Pro Pro Leu Ala Gly  
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 cat cct tac aga aag aga tgt ctc gag cat gag cac tca gag agt ttc 684  
 His Pro Tyr Arg Lys Arg Cys Leu Glu His Glu His Ser Glu Ser Phe  
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MBI-20 Sequence Listing.ST25

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agg aaa aat cgg atg aag aga acc gtg aga gta ccg gcg ata agt gca	780
Arg Lys Asn Arg Met Lys Arg Thr Val Arg Val Pro Ala Ile Ser Ala	
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aag atc gcc gat att cca ccg gac gaa tat tcg tgg agg aag tac gga	828
Lys Ile Ala Asp Ile Pro Asp Glu Tyr Ser Trp Arg Lys Tyr Gly	
240 245 250	
caa aaa ccg atc aag ggc tca cca cac cca cgt ggt tac tac aag tgc	876
Gln Lys Pro Ile Lys Gly Ser Pro His Pro Arg Gly Tyr Tyr Lys Cys	
255 260 265 270	
agt aca ttc aga gga tgt cca gcg agg aaa cac gtg gaa cga gca tta	924
Ser Thr Phe Arg Gly Cys Pro Ala Arg Lys His Val Glu Arg Ala Leu	
275 280 285	
gat gat cca gcg atg ctt att gtg aca tac gaa gga gag cac cgt cat	972
Asp Asp Pro Ala Met Leu Ile Val Thr Tyr Glu Gly Glu His Arg His	
290 295 300	
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Asn Gln Ser Ala Met Gln Glu Asn Ile Ser Ser Ser Gly Ile Asn Asp	
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tta gtg ttt gcc tcg gct tga cttttttttg tactatttgt tttttgattt	1071
Leu Val Phe Ala Ser Ala	
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aaaa	1195

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35 40 45	
Glu Ile Thr Asp Phe Thr Val Ser Lys Phe Lys Thr Val Ile Ser Leu	
50 55 60	
Leu Asn Arg Thr Gly His Ala Arg Phe Arg Arg Gly Pro Val His Ser	
65 70 75 80	
Thr Ser Ser Ala Ala Ser Gln Lys Leu Gln Ser Gln Ile Val Lys Asn	
85 90 95	
Thr Gln Pro Glu Ala Pro Ile Val Arg Thr Thr Thr Asn His Pro Gln	
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Ile Val Pro Pro Pro Ser Ser Val Thr Leu Asp Phe Ser Lys Pro Ser	
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## MBI-20 Sequence Listing.ST25

Ile Phe Gly Thr Lys Ala Lys Ser Ala Glu Leu Glu Phe Ser Lys Glu  
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 Asn Phe Ser Val Ser Leu Asn Ser Ser Phe Met Ser Ser Ala Ile Thr  
 145 150 155 160  
 Gly Asp Gly Ser Val Ser Asn Gly Lys Ile Phe Leu Ala Ser Ala Pro  
 165 170 175  
 Ser Gln Pro Val Asn Ser Ser Gly Lys Pro Pro Leu Ala Gly His Pro  
 180 185 190  
 Tyr Arg Lys Arg Cys Leu Glu His Glu His Ser Glu Ser Phe Ser Gly  
 195 200 205  
 Lys Val Ser Gly Ser Ala Tyr Gly Lys Cys His Cys Lys Lys Arg Lys  
 210 215 220  
 Asn Arg Met Lys Arg Thr Val Arg Val Pro Ala Ile Ser Ala Lys Ile  
 225 230 235 240  
 Ala Asp Ile Pro Pro Asp Glu Tyr Ser Trp Arg Lys Tyr Gly Gln Lys  
 245 250 255  
 Pro Ile Lys Gly Ser Pro His Pro Arg Gly Tyr Tyr Lys Cys Ser Thr  
 260 265 270  
 Phe Arg Gly Cys Pro Ala Arg Lys His Val Glu Arg Ala Leu Asp Asp  
 275 280 285  
 Pro Ala Met Leu Ile Val Thr Tyr Glu Gly Glu His Arg His Asn Gln  
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 aag aaa cta act ttg att ctt ggt gta agt gga ctc tgc att ttg ttc 96  
 Lys Lys Leu Thr Leu Ile Leu Gly Val Ser Gly Leu Cys Ile Leu Phe  
 20 25 30  
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MBI-20 Sequence Listing.ST25															
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Lys	Leu	Gly	Cys	Glu	Thr	Gln	Ser	Asn	Pro	Ser	Ser	Ser	Ser	Ser	Ser
	50					55				60					
tcc	tca	tct	tca	gag	tca	gct	gaa	cta	gat	ttc	aaa	agc	cat	aat	cag
Ser	Ser	Ser	Ser	Glu	Ser	Ala	Glu	Leu	Asp	Phe	Lys	Ser	His	Asn	Gln
	65				70				75					80	
att	gag	tta	aag	gaa	aca	aac	caa	acc	att	aag	tac	ttt	gaa	cca	tgt
Ile	Glu	Leu	Lys	Glu	Thr	Asn	Gln	Thr	Ile	Lys	Tyr	Phe	Glu	Pro	Cys
				85				90					95		
gaa	tta	tct	ctc	agt	gag	tac	act	cct	tgt	gaa	gac	cga	caa	aga	gga
Glu	Leu	Ser	Leu	Ser	Glu	Tyr	Thr	Pro	Cys	Glu	Asp	Arg	Gln	Arg	Gly
			100					105					110		
aga	aga	ttc	gat	agg	aac	atg	atg	aaa	tat	aga	gaa	aga	cat	tgt	cct
Arg	Arg	Phe	Asp	Arg	Asn	Met	Met	Lys	Tyr	Arg	Glu	Arg	His	Cys	Pro
		115					120					125			
gta	aaa	gat	gag	ctt	ctt	tat	tgt	ttg	att	cct	cct	cca	cca	aac	tac
Val	Lys	Asp	Glu	Leu	Leu	Tyr	Cys	Leu	Ile	Pro	Pro	Pro	Pro	Asn	Tyr
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aag	att	cca	ttt	aaa	tgg	cca	caa	agt	aga	gac	tat	gct	tgg	tat	gac
Lys	Ile	Pro	Phe	Lys	Trp	Pro	Gln	Ser	Arg	Asp	Tyr	Ala	Trp	Tyr	Asp
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aat	atc	cct	cac	aag	gaa	ctt	agt	gtt	gag	aaa	gca	gtt	caa	aac	tgg
Asn	Ile	Pro	His	Lys	Glu	Leu	Ser	Val	Glu	Lys	Ala	Val	Gln	Asn	Trp
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att	caa	gtt	gaa	ggt	gac	cgc	ttt	aga	ttc	cct	ggt	ggt	ggt	act	atg
Ile	Gln	Val	Glu	Gly	Asp	Arg	Phe	Arg	Phe	Pro	Gly	Gly	Gly	Thr	Met
			180				185						190		
ttt	cct	cgt	gga	gct	gat	gct	tat	atc	gat	gat	att	gct	agg	ctt	att
Phe	Pro	Arg	Gly	Ala	Asp	Ala	Tyr	Ile	Asp	Asp	Ile	Ala	Arg	Leu	Ile
		195					200					205			
cct	ctt	act	gat	ggt	gga	atc	aga	aca	gct	att	gac	act	gga	tgt	ggt
Pro	Leu	Thr	Asp	Gly	Gly	Ile	Arg	Thr	Ala	Ile	Asp	Thr	Gly	Cys	Gly
	210					215					220				
gtt	gca	agt	ttt	ggt	gct	tac	ctc	ttg	aag	aga	gac	att	atg	gct	gtg
Val	Ala	Ser	Phe	Gly	Ala	Tyr	Leu	Leu	Lys	Arg	Asp	Ile	Met	Ala	Val
	225				230					235				240	
tct	ttt	gct	cca	aga	gac	act	cat	gaa	gct	cag	gta	cag	ttt	gct	tta
Ser	Phe	Ala	Pro	Arg	Asp	Thr	His	Glu	Ala	Gln	Val	Gln	Phe	Ala	Leu
			245					250					255		
gaa	cgc	gga	gtt	cct	gcg	ata	atc	ggg	att	atg	gga	tca	aga	aga	ctt
Glu	Arg	Gly	Val	Pro	Ala	Ile	Ile	Gly	Ile	Met	Gly	Ser	Arg	Arg	Leu
		260					265					270			
cct	tat	cca	gct	aga	gct	ttt	gat	ctt	gct	cat	tgt	tct	cgt	tgt	ttg
Pro	Tyr	Pro	Ala	Arg	Ala	Phe	Asp	Leu	Ala	His	Cys	Ser	Arg	Cys	Leu
		275					280					285			
atc	cct	tgg	ttt	aaa	aat	gat	ggt	ttg	tac	ctt	atg	gag	gtc	gac	cgg
Ile	Pro	Trp	Phe	Lys	Asn	Asp	Gly	Leu	Tyr	Leu	Met	Glu	Val	Asp	Arg
	290					295					300				
gtt	tta	aga	ccg	ggc	ggt	tac	tgg	atc	ctc	tcg	gga	cca	ccg	att	aac
Val	Leu	Arg	Pro	Gly	Gly	Tyr	Trp	Ile	Leu	Ser	Gly	Pro	Pro	Ile	Asn
	305				310					315				320	
tgg	aaa	cag	tac	tgg	aga	ggg	tgg	gag	aga	aca	gag	gag	gat	ttg	aag
Trp	Lys	Gln	Tyr	Trp	Arg	Gly	Trp	Glu	Arg	Thr	Glu	Glu	Asp	Leu	Lys
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## MBI-20 Sequence Listing.ST25

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aaa gta act gaa aaa ggt gac tta tca att tgg caa aag cct ctc aat Lys Val Thr Glu Lys Gly Asp Leu Ser Ile Trp Gln Lys Pro Leu Asn 355 360 365	1104
cac att gag tgt aaa aag ctc aaa caa aac aat aag tca cct ccg ata His Ile Glu Cys Lys Lys Leu Lys Gln Asn Asn Lys Ser Pro Pro Ile 370 375 380	1152
tgc agc tca gat aac gcg gat tcc gct tgg tac aaa gac ttg gaa act Cys Ser Ser Asp Asn Ala Asp Ser Ala Trp Tyr Lys Asp Leu Glu Thr 385 390 395 400	1200
tgt ata aca cca tta cca gaa aca aac aat cca gat gat tca gca ggc Cys Ile Thr Pro Leu Pro Glu Thr Asn Asn Pro Asp Asp Ser Ala Gly 405 410 415	1248
ggc gca ctc gag gat tgg cca gac cga gca ttc gcg gta cct cca aga Gly Ala Leu Glu Asp Trp Pro Asp Arg Ala Phe Ala Val Pro Pro Arg 420 425 430	1296
atc atc aga gga act ata cca gaa atg aac gcg gag aaa ttt aga gaa Ile Ile Arg Gly Thr Ile Pro Glu Met Asn Ala Glu Lys Phe Arg Glu 435 440 445	1344
gac aac gag gtt tgg aaa gag aga ata gca cat tac aag aag ata gtc Asp Asn Glu Val Trp Lys Glu Arg Ile Ala His Tyr Lys Lys Ile Val 450 455 460	1392
ccg gag ctt tca cat gga aga ttc agg aac att atg gac atg aac gct Pro Glu Leu Ser His Gly Arg Phe Arg Asn Ile Met Asp Met Asn Ala 465 470 475 480	1440
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atg aac gtt gtc ccg gtc gat gca gag aaa caa acg tta ggt gtg atc Met Asn Val Val Pro Val Asp Ala Glu Lys Gln Thr Leu Gly Val Ile 500 505 510	1536
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tca acg tat cca aga act tat gat atg att cat gca gga gga ttg ttc Ser Thr Tyr Pro Arg Thr Tyr Asp Met Ile His Ala Gly Gly Leu Phe 530 535 540	1632
agc tta tac gaa cat agg tgt gat ttg acg ttg ata ttg ttg gag atg Ser Leu Tyr Glu His Arg Cys Asp Leu Thr Leu Ile Leu Leu Glu Met 545 550 555 560	1680
gat cga att ttg aga cca gaa gga aca gtt gtg ttg aga gat aat gtg Asp Arg Ile Leu Arg Pro Glu Gly Thr Val Val Leu Arg Asp Asn Val 565 570 575	1728
gag acg ttg aat aag gta gag aag ata gtg aag gga atg aag tgg aag Glu Thr Leu Asn Lys Val Glu Lys Ile Val Lys Gly Met Lys Trp Lys 580 585 590	1776
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ctt gtt gct gtt aaa act tat tgg act ggt caa cct tct gac aag aac Leu Val Ala Val Lys Thr Tyr Trp Thr Gly Gln Pro Ser Asp Lys Asn 610 615 620	1872
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## MBI-20 Sequence Listing.ST25

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Tyr Val Leu Gly Ala Trp Gln Ala Asn Thr Val Pro Ser Ser Ile Ser
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Lys Leu Gly Cys Glu Thr Gln Ser Asn Pro Ser Ser Ser Ser Ser
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Ser Ser Ser Ser Glu Ser Ala Glu Leu Asp Phe Lys Ser His Asn Gln
65          70          75          80

Ile Glu Leu Lys Glu Thr Asn Gln Thr Ile Lys Tyr Phe Glu Pro Cys
85          90          95

Glu Leu Ser Leu Ser Glu Tyr Thr Pro Cys Glu Asp Arg Gln Arg Gly
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Arg Arg Phe Asp Arg Asn Met Met Lys Tyr Arg Glu Arg His Cys Pro
115         120         125

Val Lys Asp Glu Leu Leu Tyr Cys Leu Ile Pro Pro Pro Pro Asn Tyr
130         135         140

Lys Ile Pro Phe Lys Trp Pro Gln Ser Arg Asp Tyr Ala Trp Tyr Asp
145         150         155         160

Asn Ile Pro His Lys Glu Leu Ser Val Glu Lys Ala Val Gln Asn Trp
165         170         175

Ile Gln Val Glu Gly Asp Arg Phe Arg Phe Pro Gly Gly Gly Thr Met
180         185         190

Phe Pro Arg Gly Ala Asp Ala Tyr Ile Asp Asp Ile Ala Arg Leu Ile
195         200         205

Pro Leu Thr Asp Gly Gly Ile Arg Thr Ala Ile Asp Thr Gly Cys Gly
210         215         220

Val Ala Ser Phe Gly Ala Tyr Leu Leu Lys Arg Asp Ile Met Ala Val
225         230         235         240

Ser Phe Ala Pro Arg Asp Thr His Glu Ala Gln Val Gln Phe Ala Leu
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Glu Arg Gly Val Pro Ala Ile Ile Gly Ile Met Gly Ser Arg Arg Leu
260         265         270

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## MBI-20 Sequence Listing.ST25

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 Trp Lys Gln Tyr Trp Arg Gly Trp Glu Arg Thr Glu Glu Asp Leu Lys  
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 Lys Glu Gln Asp Ser Ile Glu Asp Val Ala Lys Ser Leu Cys Trp Lys  
 340 345 350  
 Lys Val Thr Glu Lys Gly Asp Leu Ser Ile Trp Gln Lys Pro Leu Asn  
 355 360 365  
 His Ile Glu Cys Lys Lys Leu Lys Gln Asn Asn Lys Ser Pro Pro Ile  
 370 375 380  
 Cys Ser Ser Asp Asn Ala Asp Ser Ala Trp Tyr Lys Asp Leu Glu Thr  
 385 390 395 400  
 Cys Ile Thr Pro Leu Pro Glu Thr Asn Asn Pro Asp Asp Ser Ala Gly  
 405 410 415  
 Gly Ala Leu Glu Asp Trp Pro Asp Arg Ala Phe Ala Val Pro Pro Arg  
 420 425 430  
 Ile Ile Arg Gly Thr Ile Pro Glu Met Asn Ala Glu Lys Phe Arg Glu  
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 Asp Asn Glu Val Trp Lys Glu Arg Ile Ala His Tyr Lys Lys Ile Val  
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 Pro Glu Leu Ser His Gly Arg Phe Arg Asn Ile Met Asp Met Asn Ala  
 465 470 475 480  
 Phe Leu Gly Gly Phe Ala Ala Ser Met Leu Lys Tyr Pro Ser Trp Val  
 485 490 495  
 Met Asn Val Val Pro Val Asp Ala Glu Lys Gln Thr Leu Gly Val Ile  
 500 505 510  
 Tyr Glu Arg Gly Leu Ile Gly Thr Tyr Gln Asp Trp Cys Glu Gly Phe  
 515 520 525  
 Ser Thr Tyr Pro Arg Thr Tyr Asp Met Ile His Ala Gly Gly Leu Phe  
 530 535 540  
 Ser Leu Tyr Glu His Arg Cys Asp Leu Thr Leu Ile Leu Leu Glu Met  
 545 550 555 560  
 Asp Arg Ile Leu Arg Pro Glu Gly Thr Val Val Leu Arg Asp Asn Val

## MBI-20 Sequence Listing.ST25

565

570

575

Glu Thr Leu Asn Lys Val Glu Lys Ile Val Lys Gly Met Lys Trp Lys  
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Ser Gln Ile Val Asp His Glu Lys Gly Pro Phe Asn Pro Glu Lys Ile  
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tttcccacaa atttcaactc ttgttctctt catccaaagt aaaaaacaaa tcgttgcaag 180  
tgagggttgg ttttggtggt atagaatt atg aag agc ggg aag caa tct tcg 232  
Met Lys Ser Gly Lys Gln Ser Ser  
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caa cct gaa aag ggt act tcc agg atc ttg tca ctg act gtc ctg ttt 280  
Gln Pro Glu Lys Gly Thr Ser Arg Ile Leu Ser Leu Thr Val Leu Phe  
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atc gca ttt tgc ggt ttc tcc ttc tac ctc ggt ggt ata ttt tgc tct 328  
Ile Ala Phe Cys Gly Phe Ser Phe Tyr Leu Gly Gly Ile Phe Cys Ser  
25 30 35 40  
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Glu Arg Asp Lys Ile Val Ala Lys Asp Val Thr Arg Thr Thr Lys  
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Ala Val Ala Ser Pro Lys Glu Pro Thr Ala Thr Pro Ile Gln Ile Lys  
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Ser Val Ser Phe Pro Glu Cys Gly Ser Glu Phe Gln Asp Tyr Thr Pro  
75 80 85  
tgc acc gat cca aag agg tgg aag aag tat ggt gtc cat cgc tta agt 520  
Cys Thr Asp Pro Lys Arg Trp Lys Lys Tyr Gly Val His Arg Leu Ser  
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Phe Leu Glu Arg His Cys Pro Pro Val Tyr Glu Lys Asn Glu Cys Leu  
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Ile Pro Pro Pro Asp Gly Tyr Lys Pro Pro Ile Arg Trp Pro Lys Ser  
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cga gaa cag tgt tgg tac agg aac gtg cct tat gat tgg atc aat aag 664  
Arg Glu Gln Cys Trp Tyr Arg Asn Val Pro Tyr Asp Trp Ile Asn Lys

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ttc cct ggt ggt ggt acc atg ttc cct cgt gga gtt agt cac tat gtt	Phe Pro Gly Gly Gly Thr Met Phe Pro Arg Gly Val Ser His Tyr Val	760																
170						175						180						
gat ttg atg caa gat ctg att cct gaa atg aaa gac gga aca gtc agg	Asp Leu Met Gln Asp Leu Ile Pro Glu Met Lys Asp Gly Thr Val Arg	808																
185						190						195						
acc gcc att gat act ggc tgt ggg gtt gcg agc tgg gga ggc gat ctt	Thr Ala Ile Asp Thr Gly Cys Gly Val Ala Ser Trp Gly Gly Asp Leu	856																
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ttg gac cgt ggg ata cta tca ctc tct ctt gct cca aga gat aac cat	Leu Asp Arg Gly Ile Leu Ser Leu Ser Leu Ala Pro Arg Asp Asn His	904																
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235						240						245						
ggg atc atc tct acg caa cgt ctc cct ttt cct tca aat gca ttt gat	Gly Ile Ile Ser Thr Gln Arg Leu Pro Phe Pro Ser Asn Ala Phe Asp	1000																
250						255						260						
atg gct cat tgt tca aga tgt ctt att ccc tgg aca gaa ttt ggt gga	Met Ala His Cys Ser Arg Cys Leu Ile Pro Trp Thr Glu Phe Gly Gly	1048																
265						270						275						
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aac aca acc atg gaa gat cag aaa tct gac tac aac aag ctt cag tca	Asn Thr Thr Met Glu Asp Gln Lys Ser Asp Tyr Asn Lys Leu Gln Ser	1192																
315						320						325						
ctt cta acc tcc atg tgt ttc aaa aag tac gct caa aaa gat gac ata	Leu Leu Thr Ser Met Cys Phe Lys Lys Tyr Ala Gln Lys Asp Asp Ile	1240																
330						335						340						
gcc gtg tgg cag aaa ctc tca gac aaa tct tgc tat gac aaa atc gct	Ala Val Trp Gln Lys Leu Ser Asp Lys Ser Cys Tyr Asp Lys Ile Ala	1288																
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cct aaa gtc aag aag tct ggt ctc gga tca atc cca aaa tgg ccc gag	Pro Lys Val Lys Lys Ser Gly Leu Gly Ser Ile Pro Lys Trp Pro Glu	1432																
395						400						405						
agg tta cat gtc gcg ccc gag aga atc ggt gat gtt cac gga ggg agt	Arg Leu His Val Ala Pro Glu Arg Ile Gly Asp Val His Gly Gly Ser	1480																
410						415						420						
gcg aac agt ttg aaa cac gat gat ggt aaa tgg aag aac aga gtt aag	Ala Asn Ser Leu Lys His Asp Asp Gly Lys Trp Lys Asn Arg Val Lys	1528																
425						430						435						
cat tac aag aaa gtt tta cca gct ctt ggg aca gac aag ata aga aat		1576																



## MBI-20 Sequence Listing.ST25

His Tyr Lys Lys Val Leu Pro Ala Leu Gly Thr Asp Lys Ile Arg Asn  
 445 450 455  
 gtt atg gat atg aac act gtt tat gga ggt ttc tct gcg gcc ctc att 1624  
 Val Met Asp Met Asn Thr Val Tyr Gly Gly Phe Ser Ala Ala Leu Ile  
 460 465 470  
 gag gat ccc att tgg gtc atg aac gtt gta tca tcg tac agc gca aat 1672  
 Glu Asp Pro Ile Trp Val Met Asn Val Val Ser Ser Tyr Ser Ala Asn  
 475 480 485  
 tcg ctt cct gtt gtc ttt gat cgc ggt ctc atc ggg act tac cac gac 1720  
 Ser Leu Pro Val Val Phe Asp Arg Gly Leu Ile Gly Thr Tyr His Asp  
 490 495 500  
 tgg tgc gaa gct ttc tca acg tat cca aga aca tat gat ctt ctt cac 1768  
 Trp Cys Glu Ala Phe Ser Thr Tyr Pro Arg Thr Tyr Asp Leu Leu His  
 505 510 515 520  
 ctc gac agt ctt ttt acc ttg gag agt cac agg tgt gag atg aag tac 1816  
 Leu Asp Ser Leu Phe Thr Leu Glu Ser His Arg Cys Glu Met Lys Tyr  
 525 530 535  
 att ttg cta gag atg gac agg atc ttg cgg ccg agt gga tat gtt ata 1864  
 Ile Leu Leu Glu Met Asp Arg Ile Leu Arg Pro Ser Gly Tyr Val Ile  
 540 545 550  
 atc cga gaa tcg agt tat ttc atg gac gca atc aca acg tta gcg aaa 1912  
 Ile Arg Glu Ser Ser Tyr Phe Met Asp Ala Ile Thr Thr Leu Ala Lys  
 555 560 565  
 ggg ata agg tgg agt tgc cgg aga gag gag act gag tat gca gtc aaa 1960  
 Gly Ile Arg Trp Ser Cys Arg Arg Glu Glu Thr Glu Tyr Ala Val Lys  
 570 575 580  
 agt gag aag att ctg gtt tgc cag aaa aag cta tgg ttt tcg tca aac 2008  
 Ser Glu Lys Ile Leu Val Cys Gln Lys Lys Leu Trp Phe Ser Ser Asn  
 585 590 595 600  
 caa acc tct tga tgagaccacc tgtatcatag tgtttatcat ctctgtgat 2060  
 Gln Thr Ser  
 gcacactaca gagagaagga tctagtcctt tgagtccaag atatagctct ataaacaatc 2120  
 tcctttttttt gttctcttta atttcttggg tatttcacgg tatagattga tattatatat 2180  
 tttttaatta tatttttaaat atatagatat attagtatgt ggtttaaaca ctattattat 2240  
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 Tyr Leu Gly Gly Ile Phe Cys Ser Glu Arg Asp Lys Ile Val Ala Lys  
 35 40 45  
 Asp Val Thr Arg Thr Thr Thr Lys Ala Val Ala Ser Pro Lys Glu Pro  
 50 55 60

## MBI-20 Sequence Listing.ST25

Thr Ala Thr Pro Ile Gln Ile Lys Ser Val Ser Phe Pro Glu Cys Gly  
 65 70 75 80  
 Ser Glu Phe Gln Asp Tyr Thr Pro Cys Thr Asp Pro Lys Arg Trp Lys  
 85 90 95  
 Lys Tyr Gly Val His Arg Leu Ser Phe Leu Glu Arg His Cys Pro Pro  
 100 105 110  
 Val Tyr Glu Lys Asn Glu Cys Leu Ile Pro Pro Pro Asp Gly Tyr Lys  
 115 120 125  
 Pro Pro Ile Arg Trp Pro Lys Ser Arg Glu Gln Cys Trp Tyr Arg Asn  
 130 135 140  
 Val Pro Tyr Asp Trp Ile Asn Lys Gln Lys Ser Asn Gln His Trp Leu  
 145 150 155 160  
 Lys Lys Glu Gly Asp Lys Phe His Phe Pro Gly Gly Gly Thr Met Phe  
 165 170 175  
 Pro Arg Gly Val Ser His Tyr Val Asp Leu Met Gln Asp Leu Ile Pro  
 180 185 190  
 Glu Met Lys Asp Gly Thr Val Arg Thr Ala Ile Asp Thr Gly Cys Gly  
 195 200 205  
 Val Ala Ser Trp Gly Gly Asp Leu Leu Asp Arg Gly Ile Leu Ser Leu  
 210 215 220  
 Ser Leu Ala Pro Arg Asp Asn His Glu Ala Gln Val Gln Phe Ala Leu  
 225 230 235 240  
 Glu Arg Gly Ile Pro Ala Ile Leu Gly Ile Ile Ser Thr Gln Arg Leu  
 245 250 255  
 Pro Phe Pro Ser Asn Ala Phe Asp Met Ala His Cys Ser Arg Cys Leu  
 260 265 270  
 Ile Pro Trp Thr Glu Phe Gly Gly Ile Tyr Leu Leu Glu Ile His Arg  
 275 280 285  
 Ile Val Arg Pro Gly Gly Phe Trp Val Leu Ser Gly Pro Pro Val Asn  
 290 295 300  
 Tyr Asn Arg Arg Trp Arg Gly Trp Asn Thr Thr Met Glu Asp Gln Lys  
 305 310 315 320  
 Ser Asp Tyr Asn Lys Leu Gln Ser Leu Leu Thr Ser Met Cys Phe Lys  
 325 330 335  
 Lys Tyr Ala Gln Lys Asp Asp Ile Ala Val Trp Gln Lys Leu Ser Asp  
 340 345 350  
 Lys Ser Cys Tyr Asp Lys Ile Ala Lys Asn Met Glu Ala Tyr Pro Pro

MBI-20 Sequence Listing.ST25  
360 365

355

Lys Cys Asp Asp Ser Ile Glu Pro Asp Ser Ala Trp Tyr Thr Pro Leu  
370 375 380

Arg Pro Cys Val Val Ala Pro Thr Pro Lys Val Lys Lys Ser Gly Leu  
385 390 395 400

Gly Ser Ile Pro Lys Trp Pro Glu Arg Leu His Val Ala Pro Glu Arg  
405 410 415

Ile Gly Asp Val His Gly Gly Ser Ala Asn Ser Leu Lys His Asp Asp  
420 425 430

Gly Lys Trp Lys Asn Arg Val Lys His Tyr Lys Lys Val Leu Pro Ala  
435 440 445

Leu Gly Thr Asp Lys Ile Arg Asn Val Met Asp Met Asn Thr Val Tyr  
450 455 460

Gly Gly Phe Ser Ala Ala Leu Ile Glu Asp Pro Ile Trp Val Met Asn  
465 470 475 480

Val Val Ser Ser Tyr Ser Ala Asn Ser Leu Pro Val Val Phe Asp Arg  
485 490 495

Gly Leu Ile Gly Thr Tyr His Asp Trp Cys Glu Ala Phe Ser Thr Tyr  
500 505 510

Pro Arg Thr Tyr Asp Leu Leu His Leu Asp Ser Leu Phe Thr Leu Glu  
515 520 525

Ser His Arg Cys Glu Met Lys Tyr Ile Leu Leu Glu Met Asp Arg Ile  
530 535 540

Leu Arg Pro Ser Gly Tyr Val Ile Ile Arg Glu Ser Ser Tyr Phe Met  
545 550 555 560

Asp Ala Ile Thr Thr Leu Ala Lys Gly Ile Arg Trp Ser Cys Arg Arg  
565 570 575

Glu Glu Thr Glu Tyr Ala Val Lys Ser Glu Lys Ile Leu Val Cys Gln  
580 585 590

Lys Lys Leu Trp Phe Ser Ser Asn Gln Thr Ser  
595 600

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<223> G308

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MBI-20 Sequence Listing.ST25										
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tcacaaataa	agcaaaacct	agatccgaca	ttgaaggaaa	aaccttttag	atccatctct					180
gaaaaaaacc	caacc	atg aag aga	gat cat cat cat cat	caa gat aag						231
		Met Lys Arg Asp	His His His His His	Gln Asp Lys						
	1		5	10						
aag act atg atg atg	aat gaa gaa gac gac	ggg aac ggc	atg gat gag							279
Lys Thr Met Met Met	Asn Glu Glu Asp Asp	Gly Asn Gly Met	Asp Glu							
	15	20	25							
ctt cta gct gtt ctt	ggg tac aag gtt agg	tca tgc gaa atg	gct gat							327
Leu Leu Ala Val Leu	Gly Tyr Lys Val Arg	Ser Ser Glu Met	Ala Asp							
	30	35	40							
gtt gct cag aaa ctc	gag cag ctt gaa gtt	atg atg tct aat	gtt caa							375
Val Ala Gln Lys Leu	Glu Gln Leu Glu Val	Met Ser Asn Val	Gln							
	45	50	55	60						
gaa gac gat ctt tct	caa ctc gct act	gag act gtt cac	tat aat ccg							423
Glu Asp Asp Leu Ser	Gln Leu Ala Thr	Glu Thr Val His	Tyr Asn Pro							
	65	70	75							
gcg gag ctt tac acg	tgg ctt gat tct atg	ctc acc gac ctt	aat cct							471
Ala Glu Leu Tyr Thr	Trp Leu Asp Ser Met	Leu Thr Asp Leu	Asn Pro							
	80	85	90							
ccg tgc tct aac gcc	gag tac gat ctt aaa	gct att ccc ggt	gac gcg							519
Pro Ser Ser Asn Ala	Glu Tyr Asp Leu Lys	Ala Ile Pro Gly	Asp Ala							
	95	100	105							
att ctc aat cag ttc	gct atc gat tgc gct	tct tgc tct aac	caa ggc							567
Ile Leu Asn Gln Phe	Ala Ile Asp Ser Ala	Ser Ser Asn Gln	Gly							
	110	115	120							
ggc gga gga gat acg	tat act aca aac aag	cgg ttg aaa tgc	tca aac							615
Gly Gly Gly Asp Thr	Tyr Thr Thr Asn Lys	Arg Leu Lys Cys	Ser Asn							
	125	130	135	140						
ggc gtc gtg gaa acc	acc aca gcg acg gct	gag tca act cgg	cat gtt							663
Gly Val Val Glu Thr	Thr Thr Thr Ala Thr	Ala Glu Ser Thr	Arg His Val							
	145	150	155							
gtc ctg gtt gac tgc	cag gag aac ggt gtg	cgt ctc gtt cac	gcg ctt							711
Val Leu Val Asp Ser	Gln Glu Asn Gly Val	Arg Leu Val His	Ala Leu							
	160	165	170							
ttg gct tgc gct gaa	gct gtt cag aag gag	aat ctg act gtg	gcg gaa							759
Leu Ala Cys Ala Glu	Ala Val Gln Lys Glu	Asn Leu Thr Val	Ala Glu							
	175	180	185							
gct ctg gtg aag caa	atc gga ttc tta gct	gtt tct caa atc	gga gct							807
Ala Leu Val Lys Gln	Ile Gly Phe Leu Ala	Val Ser Gln Ile	Gly Ala							
	190	195	200							
atg aga caa gtc gct	act tac ttc gcc gaa	gct ctc gcg cgg	cgg att							855
Met Arg Gln Val Ala	Thr Tyr Phe Ala Glu	Ala Leu Ala Arg	Arg Ile							
	205	210	215	220						
tac cgt ctc tct ccg	tgc cag agt cca atc	gac cac tct ctc	tcc gat							903
Tyr Arg Leu Ser Pro	Ser Gln Ser Pro Ile	Asp His Ser Leu	Ser Asp							
	225	230	235							
act ctt cag atg cac	ttc tac gag act tgt	cct tat ctc aag	ttc gct							951
Thr Leu Gln Met His	Phe Tyr Glu Thr Cys	Pro Tyr Leu Lys	Phe Ala							
	240	245	250							
cac ttc acg gcg aat	caa gcg att ctc gaa	gct ttt caa ggg	aag aaa							999
His Phe Thr Ala Asn	Gln Ala Ile Leu Glu	Ala Phe Gln Gly	Lys Lys							
	255	260	265							
aga gtt cat gtc att	gat ttc tct atg agt	caa ggt ctt caa	tgg ccg							1047

MBI-20 Sequence Listing.ST25

Arg	Val	His	Val	Ile	Asp	Phe	Ser	Met	Ser	Gln	Gly	Leu	Gln	Trp	Pro	
270						275					280					
gcg	ctt	atg	cag	gct	ctt	gcg	ctt	cga	cct	ggg	ggg	cct	cct	gtt	ttc	1095
Ala	Leu	Met	Gln	Ala	Leu	Ala	Leu	Arg	Pro	Gly	Gly	Pro	Pro	Val	Phe	
285					290					295					300	
cgg	tta	acc	gga	att	ggg	cca	ccg	gca	ccg	gat	aat	ttc	gat	tat	ctt	1143
Arg	Leu	Thr	Gly	Ile	Gly	Pro	Pro	Ala	Pro	Asp	Asn	Phe	Asp	Tyr	Leu	
				305					310					315		
cat	gaa	gtt	ggg	tgt	aag	ctg	gct	cat	tta	gct	gag	gcg	att	cac	gtt	1191
His	Glu	Val	Gly	Cys	Lys	Leu	Ala	His	Leu	Ala	Glu	Ala	Ile	His	Val	
			320					325					330			
gag	ttt	gag	tac	aga	gga	ttt	gtg	gct	aac	act	tta	gct	gat	ctt	gat	1239
Glu	Phe	Glu	Tyr	Arg	Gly	Phe	Val	Ala	Asn	Thr	Leu	Ala	Asp	Leu	Asp	
		335					340					345				
gct	tcg	atg	ctt	gag	ctt	aga	cca	agt	gag	att	gaa	tct	gtt	gcg	gtt	1287
Ala	Ser	Met	Leu	Glu	Leu	Arg	Pro	Ser	Glu	Ile	Glu	Ser	Val	Ala	Val	
		350				355					360					
aac	tct	gtt	ttc	gag	ctt	cac	aag	ctc	ttg	gga	cga	cct	ggg	gcg	atc	1335
Asn	Ser	Val	Phe	Glu	Leu	His	Lys	Leu	Leu	Gly	Arg	Pro	Gly	Ala	Ile	
					370					375					380	
gat	aag	gtt	ctt	ggg	gtg	gtg	aat	cag	att	aaa	ccg	gag	att	ttc	act	1383
Asp	Lys	Val	Leu	Gly	Val	Val	Asn	Gln	Ile	Lys	Pro	Glu	Ile	Phe	Thr	
				385					390					395		
gtg	gtt	gag	cag	gaa	tcg	aac	cat	aat	agt	ccg	att	ttc	tta	gat	cgg	1431
Val	Val	Glu	Gln	Glu	Ser	Asn	His	Asn	Ser	Pro	Ile	Phe	Leu	Asp	Arg	
			400					405					410			
ttt	act	gag	tcg	ttg	cat	tat	tac	tcg	acg	ttg	ttt	gac	tcg	ttg	gaa	1479
Phe	Thr	Glu	Ser	Leu	His	Tyr	Tyr	Ser	Thr	Leu	Phe	Asp	Ser	Leu	Glu	
		415					420					425				
ggg	gta	ccg	agt	ggg	caa	gac	aag	gtc	atg	tcg	gag	gtt	tac	ttg	ggg	1527
Gly	Val	Pro	Ser	Gly	Gln	Asp	Lys	Val	Met	Ser	Glu	Val	Tyr	Leu	Gly	
		430				435					440					
aaa	cag	atc	tgc	aac	gtt	gtg	gct	tgt	gat	gga	cct	gac	cga	gtt	gag	1575
Lys	Gln	Ile	Cys	Asn	Val	Val	Ala	Cys	Asp	Gly	Pro	Asp	Arg	Val	Glu	
		445			450					455					460	
cgt	cat	gaa	acg	ttg	agt	cag	tgg	agg	aac	cgg	ttc	ggg	tct	gct	ggg	1623
Arg	His	Glu	Thr	Leu	Ser	Gln	Trp	Arg	Asn	Arg	Phe	Gly	Ser	Ala	Gly	
				465				470						475		
ttt	gcg	gct	gca	cat	att	ggg	tcg	aat	gcg	ttt	aag	caa	gcg	agt	atg	1671
Phe	Ala	Ala	Ala	His	Ile	Gly	Ser	Asn	Ala	Phe	Lys	Gln	Ala	Ser	Met	
			480					485					490			
ctt	ttg	gct	ctg	ttc	aac	ggc	ggg	gag	ggg	tat	cgg	gtg	gag	gag	agt	1719
Leu	Leu	Ala	Leu	Phe	Asn	Gly	Gly	Glu	Gly	Tyr	Arg	Val	Glu	Glu	Ser	
		495				500						505				
gac	ggc	tgt	ctc	atg	ttg	ggg	tgg	cac	aca	cga	ccg	ctc	ata	gcc	acc	1767
Asp	Gly	Cys	Leu	Met	Leu	Gly	Trp	His	Thr	Arg	Pro	Leu	Ile	Ala	Thr	
		510				515					520					
tcg	gct	tgg	aaa	ctc	tcc	acc	aat	tag	atggtggctc	aatgaattga						1814
Ser	Ala	Trp	Lys	Leu	Ser	Thr	Asn									
					525		530									
tctgttgaac	cggttatgat	gatagatttc	cgaccgaagc	caactaaat	cctactgttt											1874
ttccctttgt	cacttgtaa	gatcttatct	ttcattatat	taggtaattg	aaaaatttta											1934
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## MBI-20 Sequence Listing.ST25

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&lt;212&gt; PRT

&lt;213&gt; Arabidopsis thaliana

&lt;400&gt; 38

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Met Asn Glu Glu Asp Asp Gly Asn Gly Met Asp Glu Leu Leu Ala Val  
 20 25 30

Leu Gly Tyr Lys Val Arg Ser Ser Glu Met Ala Asp Val Ala Gln Lys  
 35 40 45

Leu Glu Gln Leu Glu Val Met Met Ser Asn Val Gln Glu Asp Asp Leu  
 50 55 60

Ser Gln Leu Ala Thr Glu Thr Val His Tyr Asn Pro Ala Glu Leu Tyr  
 65 70 75 80

Thr Trp Leu Asp Ser Met Leu Thr Asp Leu Asn Pro Pro Ser Ser Asn  
 85 90 95

Ala Glu Tyr Asp Leu Lys Ala Ile Pro Gly Asp Ala Ile Leu Asn Gln  
 100 105 110

Phe Ala Ile Asp Ser Ala Ser Ser Ser Asn Gln Gly Gly Gly Gly Asp  
 115 120 125

Thr Tyr Thr Thr Asn Lys Arg Leu Lys Cys Ser Asn Gly Val Val Glu  
 130 135 140

Thr Thr Thr Ala Thr Ala Glu Ser Thr Arg His Val Val Leu Val Asp  
 145 150 155 160

Ser Gln Glu Asn Gly Val Arg Leu Val His Ala Leu Leu Ala Cys Ala  
 165 170 175

Glu Ala Val Gln Lys Glu Asn Leu Thr Val Ala Glu Ala Leu Val Lys  
 180 185 190

Gln Ile Gly Phe Leu Ala Val Ser Gln Ile Gly Ala Met Arg Gln Val  
 195 200 205

Ala Thr Tyr Phe Ala Glu Ala Leu Ala Arg Arg Ile Tyr Arg Leu Ser  
 210 215 220

Pro Ser Gln Ser Pro Ile Asp His Ser Leu Ser Asp Thr Leu Gln Met  
 225 230 235 240

His Phe Tyr Glu Thr Cys Pro Tyr Leu Lys Phe Ala His Phe Thr Ala  
 245 250 255

Asn Gln Ala Ile Leu Glu Ala Phe Gln Gly Lys Lys Arg Val His Val  
 260 265 270

Ile Asp Phe Ser Met Ser Gln Gly Leu Gln Trp Pro Ala Leu Met Gln

MBI-20 Sequence Listing.ST25

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Ile Gly Pro Pro Ala Pro Asp Asn Phe Asp Tyr Leu His Glu Val Gly			
305	310	315	320
Cys Lys Leu Ala His Leu Ala Glu Ala Ile His Val Glu Phe Glu Tyr			
	325	330	335
Arg Gly Phe Val Ala Asn Thr Leu Ala Asp Leu Asp Ala Ser Met Leu			
	340	345	350
Glu Leu Arg Pro Ser Glu Ile Glu Ser Val Ala Val Asn Ser Val Phe			
	355	360	365
Glu Leu His Lys Leu Leu Gly Arg Pro Gly Ala Ile Asp Lys Val Leu			
	370	375	380
Gly Val Val Asn Gln Ile Lys Pro Glu Ile Phe Thr Val Val Glu Gln			
	385	390	395
Glu Ser Asn His Asn Ser Pro Ile Phe Leu Asp Arg Phe Thr Glu Ser			
	405	410	415
Leu His Tyr Tyr Ser Thr Leu Phe Asp Ser Leu Glu Gly Val Pro Ser			
	420	425	430
Gly Gln Asp Lys Val Met Ser Glu Val Tyr Leu Gly Lys Gln Ile Cys			
	435	440	445
Asn Val Val Ala Cys Asp Gly Pro Asp Arg Val Glu Arg His Glu Thr			
	450	455	460
Leu Ser Gln Trp Arg Asn Arg Phe Gly Ser Ala Gly Phe Ala Ala Ala			
	465	470	475
His Ile Gly Ser Asn Ala Phe Lys Gln Ala Ser Met Leu Leu Ala Leu			
	485	490	495
Phe Asn Gly Gly Glu Gly Tyr Arg Val Glu Glu Ser Asp Gly Cys Leu			
	500	505	510
Met Leu Gly Trp His Thr Arg Pro Leu Ile Ala Thr Ser Ala Trp Lys			
	515	520	525
Leu Ser Thr Asn			
530			

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aatccttttaa tctcatcttt gtttatcttt aatcaaaacc caaaatttac atgggttctt	180
gaaaatctag aagaaataaa ggaaacataa caaaaataga aagaaaaaga agcta atg	238
Met	
1	
gtc tta aat atg gag tct acc gga gaa gct gtt aga tca acc acc ggt	286
Val Leu Asn Met Glu Ser Thr Gly Glu Ala Val Arg Ser Thr Thr Gly	
5 10 15	
aac gac ggt ggt att acg gtg gtt aga tcc gac gcg ccg tca gat ttc	334
Asn Asp Gly Gly Ile Thr Val Val Arg Ser Asp Ala Pro Ser Asp Phe	
20 25 30	
cac gta gct caa aga tca gaa agc tca aac caa tct ccc acc tct gtc	382
His Val Ala Gln Arg Ser Glu Ser Ser Asn Gln Ser Pro Thr Ser Val	
35 40 45	
act cct cct cca cca cag cca tcg tct cat cac aca gct cct ccg ccg	430
Thr Pro Pro Pro Pro Gln Pro Ser Ser His His Thr Ala Pro Pro Pro	
50 55 60 65	
ctg caa att tcg acg gtg acg act acg act acg acg gcc gcg atg gaa	478
Leu Gln Ile Ser Thr Val Thr Thr Thr Thr Thr Ala Ala Met Glu	
70 75 80	
ggc atc tcc ggt gga ctg atg aag aag aag cgt gga ccg cca agg aag	526
Gly Ile Ser Gly Gly Leu Met Lys Lys Lys Arg Gly Arg Pro Arg Lys	
85 90 95	
tat gga ccg gac ggg act gtt gta gcg tta tct cct aaa ccg att tca	574
Tyr Gly Pro Asp Gly Thr Val Val Ala Leu Ser Pro Lys Pro Ile Ser	
100 105 110	
tca gcg ccg gcg ccg tcg cat ctt ccg ccg ccg agt tca cac gtc atc	622
Ser Ala Pro Ala Pro Ser His Leu Pro Pro Pro Ser Ser His Val Ile	
115 120 125	
gat ttc tcc gct tct gag aaa cgt agc aaa gtg aaa cca acg aac tcg	670
Asp Phe Ser Ala Ser Glu Lys Arg Ser Lys Val Lys Pro Thr Asn Ser	
130 135 140 145	
ttt aac aga aca aag tat cat cac caa gtt gag aat ttg ggt gaa tgg	718
Phe Asn Arg Thr Lys Tyr His His Gln Val Glu Asn Leu Gly Glu Trp	
150 155 160	
gct cct tgc tcc gtc ggt ggt aat ttc aca cct cat ata atc aca gtc	766
Ala Pro Cys Ser Val Gly Gly Asn Phe Thr Pro His Ile Ile Thr Val	
165 170 175	
aac acc ggc gag gat gta aca atg aag ata atc tcg ttt tcg caa caa	814
Asn Thr Gly Glu Asp Val Thr Met Lys Ile Ile Ser Phe Ser Gln Gln	
180 185 190	
gga cct cgc tct att tgt gtt ctg tca gca aac ggt gtt att tca agc	862
Gly Pro Arg Ser Ile Cys Val Leu Ser Ala Asn Gly Val Ile Ser Ser	
195 200 205	
gtt aca ctt cgt cag cca gat tcc tct ggc ggc aca ttg aca tac gaa	910
Val Thr Leu Arg Gln Pro Asp Ser Ser Gly Gly Thr Leu Thr Tyr Glu	
210 215 220 225	
ggc cgg ttt gag ata tta tca tta tcc ggg tca ttc atg cct aat gat	958
Gly Arg Phe Glu Ile Leu Ser Leu Ser Gly Ser Phe Met Pro Asn Asp	
230 235 240	
tca ggc gga aca cga agt aga acg gga gga atg agt gta tcg tta gca	1006



MBI-20 Sequence Listing.ST25

Ser Gly Gly Thr Arg Ser Arg Thr Gly Gly Met Ser Val Ser Leu Ala	
245 250 255	
agt ccc gat gga cgt gta gta ggc ggt ggc ctc gcc ggt tta cta gta	1054
Ser Pro Asp Gly Arg Val Val Gly Gly Gly Leu Ala Gly Leu Leu Val	
260 265 270	
gcc gcg agt ccg gtt cag gtg gtt gta gga agt ttt tta gcg ggc act	1102
Ala Ala Ser Pro Val Gln Val Val Val Gly Ser Phe Leu Ala Gly Thr	
275 280 285	
gac cat caa gat cag aaa ccg aaa aag aac aaa cat gat ttc atg ttg	1150
Asp His Gln Asp Gln Lys Pro Lys Lys Asn Lys His Asp Phe Met Leu	
290 295 300 305	
tcg agt cct acc gct gca att cct atc tct agt gca gct gat cac cgg	1198
Ser Ser Pro Thr Ala Ala Ile Pro Ile Ser Ser Ala Ala Asp His Arg	
310 315 320	
aca atc cat tcg gtc tcg tct ctt ccg gtc aat aat aat aca tgg cag	1246
Thr Ile His Ser Val Ser Ser Leu Pro Val Asn Asn Asn Thr Trp Gln	
325 330 335	
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Asn Val Thr	
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Lys Tyr Gly Pro Asp Gly Thr Val Val Ala Leu Ser Pro Lys Pro Ile	
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## MBI-20 Sequence Listing.ST25

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 Val Asn Thr Gly Glu Asp Val Thr Met Lys Ile Ile Ser Phe Ser Gln  
 180 185 190  
 Gln Gly Pro Arg Ser Ile Cys Val Leu Ser Ala Asn Gly Val Ile Ser  
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 Ser Val Thr Leu Arg Gln Pro Asp Ser Ser Gly Gly Thr Leu Thr Tyr  
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 260 265 270  
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 Arg Thr Ile His Ser Val Ser Ser Leu Pro Val Asn Asn Asn Thr Trp  
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## MBI-20 Sequence Listing.ST25

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## MBI-20 Sequence Listing.ST25

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 Ala Thr Asp Pro  
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Val Arg Cys Phe Thr Asp Asn Leu Val Leu Cys Gln Glu Cys Asp Trp  
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Trp Gly Ile Asp Leu Lys Gly Lys Lys Lys Glu Asp Asp Glu Asp Glu

MBI-20 Sequence Listing.ST25

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Pro Glu Gly Ile Asn Gly Gly Gly Ser Ile Ser Gln Pro Ser Pro Thr	195	200	205											
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## MBI-20 Sequence Listing.ST25

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Ser Pro Lys Ser Leu Ser Glu Leu Leu Asn Ala Lys Leu Arg Lys Asn  
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Cys Lys Asp Gln Thr Pro Tyr Leu Thr Cys Leu Arg Leu Asp Asn Asp  
 100 105 110

Ser Ser His Ile Gly Val Trp Gln Lys Arg Ala Gly Ser Lys Thr Ser  
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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/31344

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(7) : C12N 5/04, 5/10, 15/00, 15/09, 15/63, 15/70, 15/74, 15/82, 15/87; C07H 21/02, 21/04; A01H 1/00, 9/00, 11/00 US CL : 435/320.1, 419, 468; 536/23.1; 800/ 278, 295																																															
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/320.1, 419, 468; 536/23.1; 800/ 278, 295 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Continuation Sheet																																															
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b> <table border="1"> <thead> <tr> <th>Category *</th> <th>Citation of document, with indication, where appropriate, of the relevant passages</th> <th>Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td>X</td> <td>Database GenEmbl, Accession Number U28422, WANG et al., Arabidopsis thaliana Dna-binding protein CCAL (CCAL) mRNA, complete cds 14 January 1997.</td> <td>4-6</td> </tr> <tr> <td>---</td> <td></td> <td>-----</td> </tr> <tr> <td>Y</td> <td></td> <td>1-3, 7-13, 25-27</td> </tr> <tr> <td>X</td> <td>Database Geneseq., Accession Number V65382, THE REGENTS OF THE UNIVERSITY OF CALIFORNIA. 15 February 1999.</td> <td>4-6</td> </tr> <tr> <td>---</td> <td></td> <td>-----</td> </tr> <tr> <td>Y</td> <td></td> <td>1-3, 7-13, 25-27</td> </tr> <tr> <td>X</td> <td>Database PIR. Accession Number T02684, ROUNSLEY et al., DNA-binding protein CCA1 - Arabidopsis thaliana. 24 March 1999.</td> <td>11</td> </tr> <tr> <td>---</td> <td></td> <td>-----</td> </tr> <tr> <td>Y</td> <td></td> <td>1-10, 12-13, 25-27</td> </tr> <tr> <td>X</td> <td>Database Geneseq, Accession Number W79280, THE REGENTS OF THE UNIVERSITY OF CALIFORNIA. 15 February 1999.</td> <td>11</td> </tr> <tr> <td>---</td> <td></td> <td>-----</td> </tr> <tr> <td>Y</td> <td></td> <td>1-10, 12-13, 25-27</td> </tr> <tr> <td>X</td> <td>WO 98/48007 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 28 October 1998, pages 26-33, pages 43-46 SEQ ID NO: 3.</td> <td>1-13, 25-27</td> </tr> <tr> <td>P, Y</td> <td>RIECHMANN et al. A genomic perspective on plant transcription factors. Current Opinion in Plant Biology. October 2000, Vol. 3, No. 5, pages 423-434, especially pages 427-428.</td> <td>1-13, 25-27</td> </tr> </tbody> </table>			Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	X	Database GenEmbl, Accession Number U28422, WANG et al., Arabidopsis thaliana Dna-binding protein CCAL (CCAL) mRNA, complete cds 14 January 1997.	4-6	---		-----	Y		1-3, 7-13, 25-27	X	Database Geneseq., Accession Number V65382, THE REGENTS OF THE UNIVERSITY OF CALIFORNIA. 15 February 1999.	4-6	---		-----	Y		1-3, 7-13, 25-27	X	Database PIR. Accession Number T02684, ROUNSLEY et al., DNA-binding protein CCA1 - Arabidopsis thaliana. 24 March 1999.	11	---		-----	Y		1-10, 12-13, 25-27	X	Database Geneseq, Accession Number W79280, THE REGENTS OF THE UNIVERSITY OF CALIFORNIA. 15 February 1999.	11	---		-----	Y		1-10, 12-13, 25-27	X	WO 98/48007 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 28 October 1998, pages 26-33, pages 43-46 SEQ ID NO: 3.	1-13, 25-27	P, Y	RIECHMANN et al. A genomic perspective on plant transcription factors. Current Opinion in Plant Biology. October 2000, Vol. 3, No. 5, pages 423-434, especially pages 427-428.	1-13, 25-27
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---		-----																																													
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P, Y	RIECHMANN et al. A genomic perspective on plant transcription factors. Current Opinion in Plant Biology. October 2000, Vol. 3, No. 5, pages 423-434, especially pages 427-428.	1-13, 25-27																																													
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.																																															
* Special categories of cited documents: <table border="0"> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E" earlier application or patent published on or after the international filing date</td> <td>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td>"&amp;" document member of the same patent family</td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	"P" document published prior to the international filing date but later than the priority date claimed																																				
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention																																														
"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone																																														
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art																																														
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family																																														
"P" document published prior to the international filing date but later than the priority date claimed																																															
Date of the actual completion of the international search 08 February 2001 (08.02.2001)		Date of mailing of the international search report 07 MAR 2001																																													
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230		Authorized officer Cynthia Collins Telephone No. (703) 308-0196																																													



# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/31344

## C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RIECHMANN et al. The AP2/EREBP family of plant transcription factors. Biological Chemistry. June 1998, Vol. 379, No. 6, pages 633-646.	1-13, 25-27
A	RIECHMANN et al. MADS domain proteins in plant development. Biological Chemistry. October 1997, Vol. 378, No. 10, pages 1079-1101.	1-13, 25-27
A	HEARD et al. Evolutionary diversity of symbiotically induced nodule MADS box genes: characterization of nmlC5, a member of a novel subfamily. Molecular plant-microbe interactions: MPMI. July 1997, Vol. 10, No. 5, pages 665-676.	1-13, 25-27
A	HEARD et al. Symbiotic induction of a MADS-box gene during development of alfalfa root nodules. Proc. Natl. Acad. Sci. USA. 06 June 1995, Vol. 92, No. 12, pages 5273-5277.	1-13, 25-27

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/31344

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claim Nos.: 14  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-13, 25-27 SEQ ID NOS:1 and 2

Remark on Protest

☐  
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/31344

**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING** This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I-XXII, claim(s) 1-13 and 25-27, drawn to transgenic plants with modified biochemical characteristics, polynucleotides and vectors for producing said transgenic plants, and methods of making said transgenic plants. Applicant must elect one pair of sequences (one nucleotide sequence and its corresponding amino acid translation) per Group to be examined, *i.e.* SEQ ID NOS: 1 and 2 in Group I, SEQ ID NOS: 3 and 4 in Group II, SEQ ID NOS: 5 and 6 in Group III, etc.

Group XXIII, claim(s) 15-17, drawn to a method of identifying a factor that is modulated by or interacts with a polypeptide.

Group XXIV, claim(s) 18, drawn to a method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest.

Group XXV, claim(s) 19 and 20, drawn to an integrated system, computer, or computer readable medium.

Group XXVI, claim(s) 21-23, drawn to a method of identifying a polynucleotide sequence.

The inventions listed as Groups I-XXVI do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions listed as Groups I-XXVI do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Groups I-XXII are drawn to transgenic plants and methods of producing said plants with nucleic acid sequences. The methods of Groups I-XXII differ from each other in that they are directed to plant transformation methods and transgenic plants with structurally and functionally distinct nucleic acid sequences which encode structurally and functionally different amino acid sequences. In addition, Groups XXIII, XXIV, and XXVI are different methods from any of Groups I-XXII in that they have different method steps and different end products, and Group XXV requires a computer system. Thus, there is no single special technical feature which links the inventions of Groups I-XXVI under PCT Rule 13.2.

**Continuation of B. FIELDS SEARCHED Item 3:** STN (agricola, biosis, biotechno, biotechds, biotechabs, caba, caplus, embase, medline, uspatfull, wpids, pctfull, europatfull, japio) SEARCH TERMS: inventor names, plant transcription factor, fatty acid, chlorophyll, carotenoid; STIC sequence search for SEQ ID NOS: 1 and 2